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THE MORPHOLOGY, TRANSMISSION, AND LIFE-HISTORY OF HAEMOPROTEUS LOPHORTYX O'ROKE, A BLOOD PARASITE OF THE CALIFORNIA VALLEY QUAIL

BY

EARL C. O'ROKE

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* From the Zoological Laboratory, University of California, and the Division of Fish and Game, Department of Natural Resources, State of California.

INTRODUCTION

In December, 1927, while engaged in making a survey of parasites of game birds for the Division of Fish and Game of the State of California, the writer discovered the parasite of the blood of the California Valley Quail, which is the subject of this paper.

Since the original discovery was made in a wild bird shot near the State Game Farm at Yountville, California, where pheasants as well as native quail are being propagated for distribution throughout the State, it seemed possible that some species of exotic bird might be found to be the source of the infection. This assumption proved to be erroneous. Birds in that locality, other than the California Valley Quail, were found to be entirely free from these parasites. The quail in the breeding pens, on the other hand, proved to be infected in the same manner as the wild one on which the original discovery was made. By extending the survey to areas far removed from the Game Farm, the alternate hypothesis that the parasite was endemic only in the original host was verified.

From literature available it was evident that the parasite under consideration belonged to either the genus *Haemoproteus* or the genus *Proteosoma,* organisms which are commonly spoken of as causing bird malaria. The two genera are similar in appearance and in pigment formation but the gametocytes of *Proteosoma* tend to be more rounded than do those of *Haemoproteus.* When the blood of a bird infected with *Haemoproteus* cools after death, however, the gametocytes tend to round up and simulate those of *Proteosoma.* There is one outstanding biological difference. *Proteosoma* can be transmitted by direct blood inoculations whereas *Haemoproteus* cannot. Blood tests were therefore made, using the blood of three infected quail to inoculate three pheasants, six chicks, and six quail. All the birds that received the inoculations were young healthy birds free from blood parasites of any kind. All attempts at transmission by this method were futile. This left no doubt that the parasite was *Haemoproteus.*

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Special credit is due to Professor C. A. Kofoid under whose direction the bulk of the experimental and laboratory work has been done in the Laboratory of Zoology of the University of California.

To Mr. Eugene D. Bennet, Executive Officer of the Division of Fish and Game, Dr. H. C. Bryant, Mr. R. E. Ludlum, Mr. D. D. McLean, Dr. H. Van Roekel, Mr. August Bade, Superintendent of the State Game Farm, and Dr. K. F. Meyer, Director of Hooper Foundation for Medical Research, the writer is indebted for cordial cooperation and assistance.

Professor W. B. Herms and Professor E. C. Van Dyke of the University of California have assisted in the work with the flies, and Professor G. F. Ferris of Stanford University has identified *Lynchia hirsuta* and allowed the use of cuts for the illustrations of the fly.

METHODS OF STUDY

For diagnosis of infection, dried films stained with Wright's blood stain sufficed. This stain was also used for determining the normals for quail blood.

For making blood counts and for determining the percentage of infected blood cells, a Thoma-Zeiss haemacytometer was used, dilutions being made with Hayem's solution. The blood-counting apparatus also proved useful for estimating the normal shape of the parasites in undisturbed blood cells and for detecting hypertrophy of the blood cells produced by the gametocytes. Permanent preparations of such hypertrophied cells were obtained by diluting the blood with normal saline, fixing with 4 per cent formaldehyde and then carrying out subsequent operations in vials. Delafield's haematoxylin and haemalum were found equally good for staining such blood cells in the vials. The last step before mounting was to let the balsam thicken gradually by evaporation.

For interpreting details of structure of the gametocytes, several methods of fixation and staining were tried. The method that gave the best results was to allow the films to dry on the slide, then fix immediately with acetone-free methyl alcohol for two minutes and stain with dilute Giemsa's azur-eosin made slightly alkaline with sodium carbonate. The films were stained from three to twenty-four hours, depending upon the intensity of staining desired. Clearing with acetone and xylol and mounting in balsam did not give so clear preparations as drying the films and studying them in cedar oil without a cover slip.

For the study of the phenomena of gametogenesis and fertilization, as well as the earliest conditions of the oökinete, a drop of fresh blood was simply mounted under the cover slip and studied with the oil immersion objective as well as with the dark field apparatus. Permanent records of these phenomena were obtained by stopping the process at any time, then fixing and staining the films as before.

For demonstrating schizogony in the tissue of the lung and other organs of the quail, both smear preparations and sections were used. Alcoholic corrosive sublimate fixation followed by iron-haematoxylin or dilute Giemsa stain gave good results. Sections cut 7.5, 10, and 15 microns proved to be of suitable thickness.

For studying the stages in sporogony in the fly, the digestive tract and salivary glands were dissected out in normal saline under the binocular and examined with the oil immersion objective and No. 10 oculars. The tissues are so beautifully transparent that one misses a good deal if he fails to study the living oöcysts and sporozoites. For permanent mounts smears are preferable to sections. The sporozoites stain well with iron-haematoxylin following fixation with Schaudinn's fluid.

Haemoproteus

BRIEF HISTORY

Danilewsky (1889) first reported *Haemoproteus*-like parasites from the blood of several kinds of birds. He mentions in particular the "Pie-grieche," "rolliers," and "chouettes." The generic name *Haemoproteus* was first used by Kruse (1890). Labbé (1893) employed the name *Halteridium* for the same parasites. This was a descriptive term based upon the fact that the mature gametocytes encircle the nuclei of the blood cells. He later reached the conclusion that there was but one species, to which he restricted the name *Haemoproteus danilewsky* Grassi and Feletti. Minchen (1912) noted that the parasites of different species of birds differed in form and size and appearance, and stated that there could be little doubt that there were many species of *Haemoproteus*. He added, however, that one species might be capable of infecting several different species of birds.

Since the early nineties the blood of hundreds of birds has been examined. Wenyon (1926) lists 302 kinds of birds from which species of *Haemoproteus* have been reported. The usual custom has been to employ the generic or specific name of the parasitized bird in forming the specific name of the parasite. For example, the species occurring in the pigeon *Columba livia* is *Haemoproteus columbae*. Wenyon disapproves of this practice because practically nothing is known of the possibility of one and the same species occurring in different hosts.

DISTRIBUTION

In reviewing the literature, one is immediately confronted with the large number of records from Europe, Asia, Africa, South America, and Australia, and with the paucity of records from North America. Nearly all the North American records are those of Opie (1898), compiled from examinations of birds made by him in the vicinity of Baltimore, Maryland. Opie studied eight species of birds, and found five kinds of *Haemoproteus*. He did not give a diagnostic description of any of these and did not assign to them either specific or varietal names. Opie did not examine any quail or partridges.

From the available records it would seem that the center of distribution of the louse flies of birds is in the Old World. It is possible, however, that more thorough investigations would disclose large numbers of species of these flies in North America. It is also possible that *Haemoproteus* will be found to have a much wider distribution in North America than the records to date indicate.

Haemoproteus lophortyx O'Roke

DISTRIBUTION

Species of *Haemoproteus* have been found in over 300 different kinds of birds, a list of which may be consulted in Wenyon's (1926) Protozoology, vol. 2, pages 1354–1387. A typical example of one of the more comprehensive lists of host birds is that of Galli-Valerio (1902). He examined 101 birds belonging to 29 genera and 36 species in the Alpine regions of central Europe. With one exception, all these birds were migratory. Among them he found *Haemoproteus* in 16 genera and 18 species. Contrasted with the above records are those taken in the course of the present studies. Of birds other than quail of the genus *Lophortyx*, 170 individuals belonging to 28 genera and 36 species were examined. Of all these birds examined, only one species, *Aphelocoma californica*, the California jay, was found to be infected with *Haemoproteus*.

Only three of these birds, all from one flock, were infected. The infection was extremely light and the species of *Haemoproteus* found was not determined. The blue jays were collected near Orinda, California, just east of the Berkeley hills.

With the exception of ten species of water birds, all the birds in the above list were taken in localities commonly frequented by quail. If either of the species of *Haemoproteus* above referred to parasitizes other birds than quail, it would seem that it should have been found in some of the other birds examined.

From accounts of parasitism of pigeons by *Haemoproteus columbae* in Europe, Africa, and India, one infers that infection is more commonly found in young birds, which presumably become infected before they leave the nests. It is also reported that in chronic cases gametocytes may be entirely wanting in the blood for months at a time.

The conditions of parasitism by *Haemoproteus lophortyx* in the California Valley Quail are apparently different. In observations extending over a period of fifteen months from December, 1927, to March, 1929, there never has been a time when gametocytes could not be found in the red blood cells of adult quail. This is equally true of penned birds at the State Game Farm at Yountville, of wild ones shot in various localities in the area covered, and of penned quail kept on the campus of the University of California at Berkeley.

The quail kept at the University consisted, in part, of birds brought from the State Game Farm and of others trapped at various localities in the area covered by this report. The number on hand varied from a half dozen to thirty. In all cases the results were the same. When infection had once become chronic, gametocytes persisted in the blood. Weekly examinations of all birds were made, and in the cases of seven of these, the period of observation extended over a year's time. Only one case was discovered that was apparently an exception. Bird 40 gave positive tests from April 7, 1928, to January 8, 1929. On January 10, the first negative test was recorded. The tests continued to be negative on January 11, 15, and 21. The bird was killed on January 21. Blood from the heart was negative but mature gametocytes were found in abundance in smear preparations made from the lungs.

As far as natural conditions of infection were concerned, the summer season of 1928 at the State Game Farm was evidently different from that of the preceding year. In April of 1928, fully 60 per cent of the quail at this place were found to be infected. Many of these birds were quail that had been hatched at the Farm in the preceding year and presumably became infected on the grounds before they reached maturity. Contrasted with this situation was the condition of the young birds that were hatched at the Farm in the spring of 1928. Representative samplings of these were examined at intervals from early June, when some of the birds were only three weeks old, to November, when all the birds had reached maturity. Not a single young bird was found to be infected. In this respect, it is significant that not a single hippoboscid fly, of any species, was found about the quail at the State Game Farm during this entire time.

Among the wild quail, the first young bird found to be infected in 1928 was taken September 21, in the hills east of Berkeley, and was estimated to be about three months old. Collections made during October and November yielded several more infected young birds, which by this time had reached maturity. It is probable that young quail became infected earlier than these records would indicate.

The initial infection of young birds is closely correlated with the presence of infected flies, and the only species of hippoboscid fly, *Lynchia hirsuta* Ferris, taken on the quail during this season in the area studied, was not at all abundant. The fact that the month of August was the best collecting time for flies in this particular season may be correlated with the appearance of infected young birds a month or two later.

In addition to the birds examined at the State Game Farm at Yountville, the quail which have furnished data for this paper were collected at many other localities in California as well as on Santa Catalina Island. Parasitized birds were found wherever collections were made in the San Francisco Bay area and along the foothills of the Coast Range from Napa to San Diego. The finding of the parasite in the Gambel Quail in private aviaries at Riverside and at Altadena and in the Catalina Island Quail on Santa Catalina Island extended the host distribution of *Haemoproteus lophortyx* to all the species and subspecies of the genus *Lophortyx* which inhabit California, including Santa Catalina Island. The only places where the parasite was not found were in the Sacramento and San Joaquin valleys. Whether the distribution of the parasite is limited to the more brushy foothill areas, as the records would indicate, is problematical. It is possible that more extensive collections of quail from the great inland valleys would likewise disclose the presence of the parasite there.

Morphology of the Gametocytes

Haemoproteus lophortyx is recognized by the presence in the red blood cells of early, immature and mature male and female gametocytes. The diagnostic characteristics of each of these seen in preparations stained with Giemsa's stain are as follows:

EARLY GAMETOCYTES

Form and size.—Ovate, spherical or elongate organisms appearing as a single clear hyaline object midway between the nucleus and the periphery of the blood cell, usually toward the end. Size about 1 micron in diameter.

Nucleus.—Not fully differentiated as yet but represented by a peripheral blob of chromatin staining red. Minute karyosome often present.

Cytoplasm.—Clear, periphery darker with occasional reticular areas which stain a pale blue.

Vacuoles.—A small clear area occupying almost the entire organism and differentiating it sharply from the cytoplasm of the surrounding host cell.

Pigment granules.—Absent.

IMMATURE GAMETOCYTES

Form and size.—Cylindrical bodies oriented as a rule parallel to the long axis of the nucleus of the blood cell. One end usually slightly broader than the other, average diameter 2 microns, length up to 8 microns, curving evident when the length of the parasite exceeds that of the nucleus of the blood cell.

Nucleus.—More definitely recognizable as a distinct, centrally located structure, oval in shape, and staining red, especially around the periphery. Nuclear membrane not yet sharply defined. Size from one-fifth to one-half the length of the gametocyte. Karyosome sometimes visible.

Cytoplasm.—Varying from clear to granular, blue staining reaction more pronounced than in earlier stages.

Vacuoles.—The single vacuole now diffuse or broken up into smaller vacuolated spaces.

Pigment granules.—From two to ten in number, and first appearing at one end of the gametocyte. Groups of two to five common. May be irregularly scattered, approaching those of adult gametocyte in size.

MATURE MALE GAMETOCYTES

Form and size.—Halter-shaped, partly encircling the nucleus of the red blood cell but not in close contact with its nuclear membrane. Along the greater part of its length, parasite extends out to the periphery of the blood cell. Diameter 1½–2½ microns, length up to 18 microns when curving is taken into consideration. Both ends uniformly rounded, but the end containing the nucleus slightly broader. Instances not rare of the parasite completely filling the space formerly occupied by the cytoplasm of the host cell. In this case, both ends of the nucleus-encircling gametocyte in contact.

Nucleus.—Elongate, ovate, almost always nearer to one end of the gametocyte, the broader end being nearer to the end of the cell. In dried films nucleus stains pink. In wet films fixed with Schaudinn's fluid and stained with iron-haematoxylin, nucleus lighter colored than surrounding cytoplasm. Average size 1½ by 4½ microns, becoming diffuse and much larger, filling three-fourths of the gametocyte, just preceding gametogenesis. Karyosome usually visible.

Cytoplasm.—Pale, almost hyaline, both in dried films and in wet fixed preparations followed by iron-haematoxylin stain.

Vacuoles.—Indistinct and diffuse, often a large one near one end with a ring of pigment granules around its periphery.

Pigment granules.—Minimum number 11, maximum 39, average 19.6, with tendency to be deposited in two more or less terminal groups with scattered grains between. Shape from spherical to rod-shaped or oval. Size from .2 to .8 microns in greatest diameter. In fresh diluted blood, pigment grains of carbon black appearance, in stained preparations, brownish. In all cases granules highly refractive.

MATURE FEMALE GAMETOCYTES

Form and size.—Like the male gametocytes, halter-shaped, encircling the nucleus but not closely applied to the nuclear membrane. Greater tendency for the two ends of gametocyte to come into contact around the nucleus than in the male gametocyte, in which case parasite loses halter-shaped appearance and appears to fill entire cytoplasm of the cell. The two ends of the gametocyte more uniform in appearance than in the male gametocyte. Measurements in general same as for male gametocytes, but tendency to produce greater hypertrophy of the erythrocyte.

Nucleus.—Spherical to oval, more centrally located than in male gametocyte, average size 1½ to 2½ microns in greater diameter. Staining reactions dark pink to red. Karyosome distinct.

Cytoplasm.—Easily distinguished from that of the male gametocyte by the darker staining both in dried films and in wet-fixed preparations. Reticular appearance of cytoplasm more apparent than in male gametocyte.

Vacuoles.—Usually present, from one the size of the nucleus to two or more smaller ones irregularly distributed.

Pigment granules.—Minimum number 15, maximum 52, average number 24.25. Tendency to fall into two groups less pronounced than in male gametocytes. Scattered distribution more common. Size up to 0.6 microns. Color and refractive appearance same as in male gametocyte.

The preceding descriptions apply to blood films made up under favorable conditions with blood drawn from the wing vein and properly prepared before destructive changes have occurred. Blood that has cooled down in the tissues of a bird after death gives an entirely erroneous picture owing to rounding up of the gametocytes.

Hypertrophy of the blood cells is common when the gametocytes reach full maturity. The average distortion of the blood cells in 100 measured cells is 9 per cent in the lesser diameter and 11 per cent in the greater diameter.

When an unusually large parasitized cell is seen, and especially when there is an exceptionally large number of pigment granules, careful search will usually disclose two or more nuclei, showing that a multiple infection is present. Such infections may consist of gametocytes of the same sex or of those of opposite sexes. Gametogenesis has never been observed to take place simultaneously in both a male and a female gametocyte derived from the same erythrocyte.

The pseudopodium-like processes so often figured on gametocytes may possibly be artifacts caused by the collapsing of vacuoles during fixation and subsequent treatment of blood films. Living gametocytes studied under a variety of conditions have never revealed any pronounced pseudopodia.

COMPARISON WITH *Haemoproteus columbae*

In the absence of good descriptions and illustrations, it is not possible from the literature to identify many of the species of *Haemoproteus* that have been reported from birds. Descriptions have been based almost altogether upon host and geographic distribution, and upon the form of the gametocytes. This form may be deceptive. Furthermore, descriptions are often couched in general terms and based upon selected specimens. Such descriptions fail to take into consideration the wide range of variation of the gametocytes. Pigment grains are often characterized by such terms as few, many, large, small, and similar expressions. Better descriptive papers have been published by Anschütz (1909) and by Wasielewski and Wülker (1918).

The illustrations of the *Haemoproteus* of the Türmfalken (kestrel), *Cerchneis tinnunculus*, published by the latter are excellent.

Since *Haemoproteus columbae* Celli and Sanfelice is the best known *Haemoproteus* from birds and since it is the only species of which the complete life-history has been published, it will be used as a basis for comparison with *Haemoproteus lophortyx* described in this paper. *Haemoproteus columbae* was originally described by Celli and Sanfelice (1891). Significant contributions were later made by Aragao (1908), Acton and Knowles (1914), and Adie (1915, 1921). *Haemoproteus lophortyx* differs from this species in the following respects:

1. GEOGRAPHICAL AND HOST DISTRIBUTION. *H. columbae* has been reported from the pigeon, *Columba livia,* in Africa, Europe, India, and South America. *H. lophortyx* has been reported thus far only from quail of the genus *Lophortyx* inhabiting California in western North America.

2. MORPHOLOGICAL CHARACTERISTICS. The gametocytes of *H. lophortyx* are more slender than those of *H. columbae* and do not regularly displace the nucleus of the erythrocyte. Such displacement, however, is not rare. Also, the gametocytes of *H. lophortyx* do not hug the nucleus of the erythrocyte as closely as do those of the other species. A comparison of the sporozoites in the transmitting hosts shows those of *H. lophortyx* to be relatively more slender in proportion to their length. Also, the nucleus of the sporozoite is relatively more slender in *H. lophortyx*. Compare figures 11 and 12, plate 1. The relatively more slender structure of the microgametes of *H. lophortyx* is also to be noted. As to the nature and arrangement of the pigment granules, about all that can be said is that they have an individuality which is apparent when hundreds of specimens are studied.

3. TRANSMISSION. *H. columbae* is transmitted by *Lynchia maura, L. brunea, L. lividicolor, L. (Olfersia) capensis* and *Microlynchia fusilla,* whereas *H. lophortyx* is transmitted by *Lynchia hirsuta.*

4. PATHOGENICITY. *H. columbae* is ordinarily considered to be a benign form. Experimental work with *H. lophortyx* clearly indicates that it may cause serious injury and even fatalities.

TRANSMISSION

The first record of experimental transmission of *Haemoproteus columbae* from pigeon to pigeon is that of Sergent, Ed. and Et. (1906). The Sergents collected *Lynchia maura,* louse flies of the family Hippoboscidae, and transported them from Algiers to Paris for experimental use. Their method was to place numerous infected flies in a cage with an uninfected pigeon and make periodic examinations of the blood of the bird to discover the first appearance of gametocytes there. They found that infection appeared in the pigeon about thirty days after it had been exposed to infective bites.

In South Africa, Gonder (1915) found that the parasite could be transmitted by *Lynchia* (*Olfersia*) *capensis*. Aragao (1916) found that the parasite could be transmitted to pigeons in South America by three different species of hippoboscidae, *L. lividicolor, L. brunea,* and *Microlynchia fusilla.* In India, Adie (1915) effected transmission with *L. maura.*

NOTES ON THE BIOLOGY OF *Lynchia hirsuta* Ferris

With this background it seemed probable that hippoboscid flies would be found to be associated with the California Valley Quail, and that they would prove to be the transmitting host of the species of *Haemoproteus* found in the bird.

A survey of museum collections of hippoboscid flies from the quail yielded few records but their very paucity demonstrated their inestimable value. If, under actual field conditions, the number of species of louse flies parasitic on the quail proved to be as scarce as the records would indicate, the solution of the problem of transmission would not involve wide trial and error experimentation. The University collections at Berkeley contained only two flies of the species *Stilbometopa impressa* Bigot taken on *Lophortyx californica.* Those of Professor G. F. Ferris, of Stanford University, contained three specimens of *Lynchia hirsuta* Ferris. Two of these were taken from quail taken at Stanford University and the third was from the brown thrasher, *Toxostoma redivivum redivivum* from Altadena. *Lynchia hirsuta* Ferris had only recently been described by Ferris (1927).

Search was therefore instituted for these and any other species of hippoboscids that might be found on quail. Nothing was known of the life-history of any of these flies in California excepting that the pupipara deposit a pupa instead of an egg and that there is no larval stage outside of the body of the insect. This abbreviated life-cycle eliminates automatically the search for eggs and larvae and enables the collector to concentrate his attention on the adults and pupae. From Adie's experiences one might expect to find both the adults and pupae around places where the birds congregate.

Conditions at the State Game Farm at Yountville, California, appeared to be favorable for finding the flies. At least 200 quail were kept there in outdoor pens throughout the year. Within the pens was natural cover for the quail, which one might expect would afford suitable places for the flies to deposit their pupae. Furthermore, many of the quail there were known to be parasitized with *Haemoproteus lophortyx.* Intensive search was made there from April to June. In addition to searching for pupae repeated examinations of the quail were made in the hope of finding the flies. The methods used involved constructing cloth-screened pens into which quail were driven for examination. Sometimes the screen was closed immediately after the quail had entered the pens, and at other times the screen was left open for a while to allow the flies to enter and settle on the quail in case they had been driven off during the process of penning the birds. This procedure was tried both in the daytime and at night, but no flies were ever found.

Operations were then transferred to the field. In the meantime the quail breeding season was about over and occasional quail were shot for examination. The first fly discovered was seen on a quail that had been shot near Mount Hamilton on June 8. Unfortunately this fly escaped and it was not identified. Better methods of collecting yielded better results but it was not until July 7 that the second fly was obtained from a quail shot near Mount Diablo. The successful method of obtaining flies was to shoot a quail in an open clearing where it could be reached in a few seconds. It was then placed immediately in a gauze bag which was closed with a rubber band. These bags were made up in convenient sizes and were conical in shape. After a quail had been placed in a bag, the bag was suspended and any flies present invariably crawled up into the tapering end. They were then collected in a vial, 22-mm. shell vials proving to be of suitable size.

By November this method of collecting had yielded thirty-two flies. *Lynchia hirsuta* Ferris was the only species found on the quail. The greatest number of flies found on any one quail was four. Some had only one or two and many had none. The time of day when the bird was shot seemed to have nothing to do with the presence or absence of flies. Brushy areas in the foothills did, however, yield flies when adjacent areas in the open valleys did not. In addition to the successful methods outlined above, modifications of the unsuccessful methods tried at the State Game Farm were continued in the field. One of these methods consisted of trapping quail in a sparrow trap and then covering the trap with gauze before examining the bird. Another method was to place a captive quail in a wire cage for hours at a time near the feeding, watering, and dusting places of the quail in their native haunts in the hope of capturing flies. An interesting modification of this method was to place two cages of such captive birds near each other but screened from view by bushes or other obstruction. The calls of these captive quail then attracted some of the wild

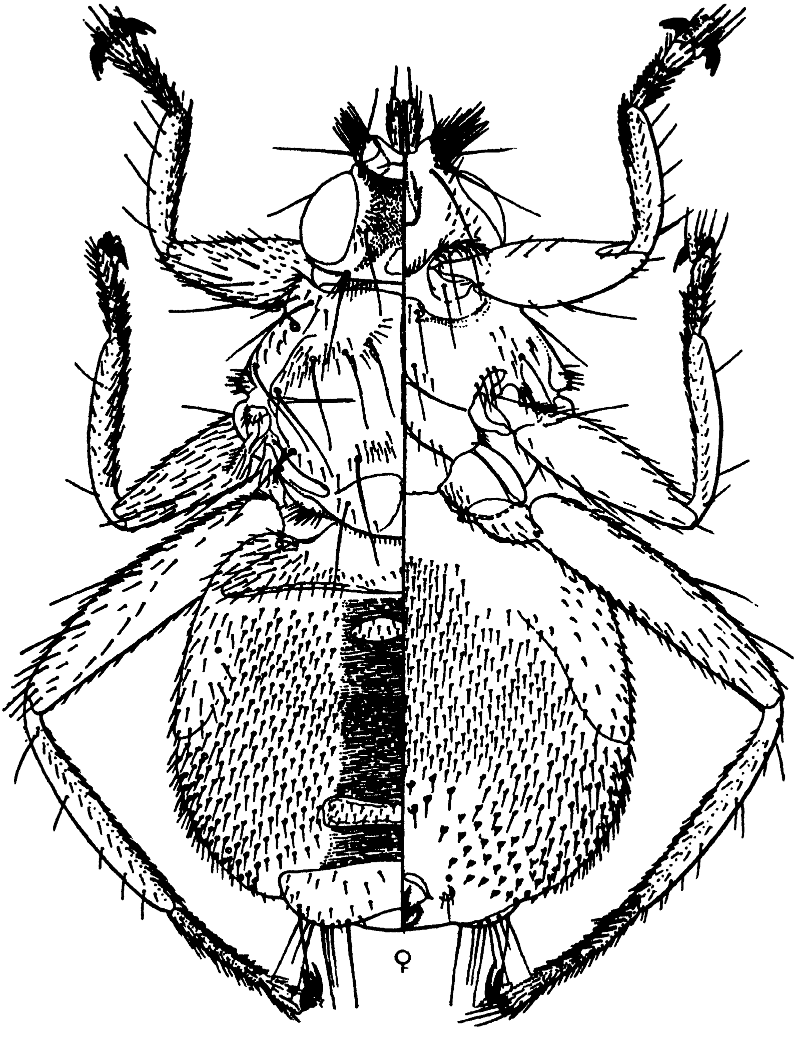

Fig. A. *Lynchia hirsuta* Ferris. Female, wings removed. After Ferris, × about 25.

ones. None of these methods yielded any flies. No pupae or flies were ever taken apart from their hosts and no pupae were found excepting those that were oviposited in the vials by captive flies. Flies were taken in every month from June to October. Hunters have reported seeing flies on quail during December and January. It is probable, therefore, that *Lynchia hirsuta* is present on its host throughout the year. Three different flies have been observed to oviposit a pupa each in a vial immediately after being captured. These ovipositions took place on July 7, August 2, and October 5. All of them were placed in a constant temperature box maintained at 35.5° C. The one oviposited on August 2 gave rise to a fly in thirty-one days. The others failed to hatch. A pair of flies were observed in copula October 5. The longest time that any fly lived in captivity was five days.

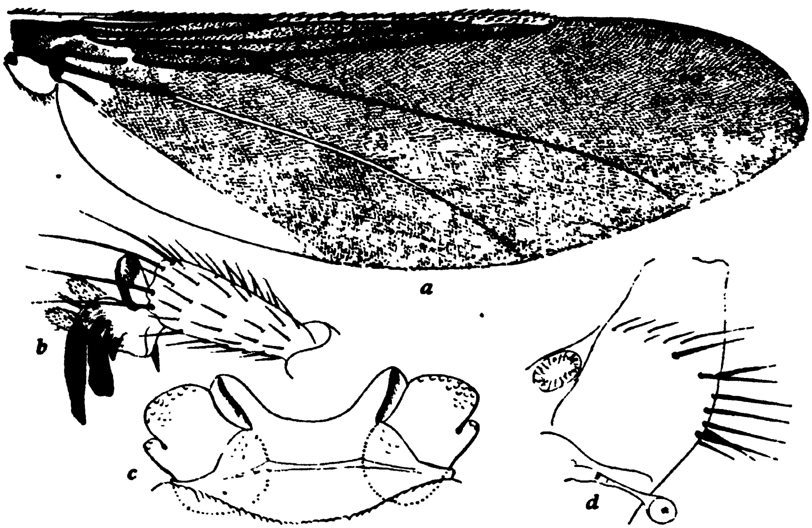

Fig. B. *Lynchia hirsuta* Ferris. *a*, wings; *b*, last segment of tarsus; *c*, clypeal region; *d*, pleurotergite of post-scutellum as seen from the ventral aspect with posterior coxa removed. After Ferris, *a*, × about 25; *b*, *c*, and *d*, × 100.

EXPERIMENTS WITH THE HIPPOBOSCID FLY *Lynchia hirsuta* Ferris

Owing to the scarcity of flies and the difficulty of obtaining them, the best possible use had to be made of all that were collected. Some had to be preserved for records, some dissected or sectioned to determine whether they were parasitized, and some used in the transmitting experiments. Losses due to accident or injury were guarded against but could not be prevented entirely. The following flies were not actually used in experiments but some notes on them are included for records.

July 20, fly 13, uninfected, was taken on quail 69, uninfected, near Mount Diablo. July 20, fly 14, uninfected, was taken on quail 71, infected, near Mount Diablo. July 30, flies 17, 18, and 19, condition of infection unknown, were taken on quail 72, infected, near Orinda. August 2, flies 20 and 21, uninfected, were taken on quail 74, infected, near Mount Diablo. Fly 20 oviposited a pupa immediately after being placed in a vial. This fly died four hours after ovipositing the pupa. A fly emerged from this pupa 31 days later. September 30, fly 29, uninfected, was taken on quail 92, an uninfected bird collected near Mount Diablo.

Unless otherwise specified, all the quail used in the fly biting experiments were young healthy birds that had been hatched on May 22. From the time of hatching they had been kept at the Game Farm in sunny outdoor pens, screened to protect the quail from any possible contacts with hippoboscid flies. Of these young quail, numbering 26 in all and confined in two different pens, none became infected while they were being kept as controls and for later experimental use.

It will be noticed that improved methods designed to conserve the material and to provide for better controls were developed as it became apparent that large numbers of flies could not be obtained. A complete account of the experiments follows:

Experiment 1, July 7. Two young quail, B5 and B6, were placed in a screened cage with flies 2 and 3 taken from quail 63, an infected bird.

July 8, one of the flies escaped when the cage was opened for feeding and caring for the birds.

July 9, the other fly was found dead on the floor of the cage. It was dissected and proved to be uninfected.

Results negative.

Experiment 2, July 7. A young quail, B7, was placed in a screened cage with quail 32, an infected bird. Four flies, 4, 5, 6, and 7, from quail 64, an infected quail, were placed in the cage.

July 8, the cage was opened inside a larger screened cage and the quail transferred individually to cloth bags for the purpose of recovering the flies. Two flies were recovered from quail 32. The other two could not be found. The abdomen of one of these flies was full, showing that it had fed recently. The quail and flies were replaced in the cage.

July 9, one dead fly was recovered from the floor of the cage. It was dissected and found to be uninfected.

July 11, the quail were examined again and one fly was recovered. It is not known from which bird it came. This fly was killed, fixed, and sectioned. It was not infected.

Results negative.

Experiment 3, July 12. A young quail, B8, was taken into the field in a screened cage. Fly 8 was obtained from quail 65, an uninfected wild bird shot at 5:30 A.M. This fly was placed in a vial and left undisturbed for two hours. The vial was then opened and placed directly against the skin of the quail. In four successive trials at intervals of half an hour the fly did not feed. This fly was taken to the laboratory and preserved for a record. The quail escaped during the last attempted feeding experiment.

Results negative.

Experiment 4, July 12. Quail B9 was taken into the field in a screened cage. Fly 9 was obtained from quail 66, an uninfected bird. Four attempts at intervals of one hour beginning at 9:00 o'clock to get this fly to feed on the quail failed. The fly was preserved for a record.

Results negative

Experiment 5, July 12. Flies 10, 11, and 12 were obtained from quail 67, an infected bird shot at 10:30 A.M. These flies were kept in a vial until 4:30 P.M. before being used in attempted feeding experiments. They failed to feed. The procedure was the same as in the preceding experiments except that the flies had been isolated longer from their host.

Results negative.

Experiment 6, July 12. All the flies used in the preceding experiment were placed in a screened cage with quail B9. This bird was kept in the cage until July 20, but the flies were not seen again.

Results negative.

Experiment 7, July 20. Flies 15 and 16 obtained from quail 71, an infected bird, were placed in a screened cage with quail B10.

July 21, one of these flies was recovered from the cage and preserved for a record. The other one had disappeared.

Results negative.

Experiment 8, August 2. Fly 22 was obtained from quail 76, an infected bird shot at 10:00 A.M. near Mount Diablo. This fly was confined in a vial until 6:00 P.M., when it was placed in a paper biting cup having a gauze top and bottom, and fastened against the skin of quail B13. The cup was attached to the feathers of the bird by means of gummed paper tape.

August 3, the fly was alive and active. Its abdomen was swollen, showing that it had fed recently. The fly was removed from the bird at 10:00 A.M., and kept warm until 5:00 P.M., when it was again placed on the bird.

August 4, the fly was alive and active. There was no evidence that it had fed again. It was then removed from the bird at 11:00 A.M. and kept warm as before. At 5:00 P.M., it was placed on quail 32, a bird harboring a chronic *Haemoproteus* infection.

August 5, 11:00 A.M., the fly was alive and active. There was no evidence that it had fed recently. The cup was again sealed against the skin of the bird.

August 6, 8:00 A.M. the fly was alive but not active. At 2:30 P.M. it was found dead in the cup. It was then dissected. The digestive tract contained digested blood and a few undigested red blood cells. There were no parasitized erythrocytes present, and no oöcysts or sporozoites could be found.

Results negative.

Experiment 9, August 10. Fly 23 was obtained from quail 84, an uninfected bird shot near Grizzly Peak east of Berkeley. It was placed in a biting cup on quail 32, the bird used in the preceding experiment.

August 11, the fly was alive and in good condition but there was no evidence that it had fed recently.

August 12, the biting cup had been torn open by the quail and the fly had disappeared.

Results negative.

Experiment 10, September 3. Flies 24 and 25 were taken respectively on quails 84 and 85, both infected birds, shot near Grizzly Peak about 7:00 P.M. Each fly was placed in a separate biting cup and sealed against the skin of quail 32, the infected bird that had been used in previous experiments.

September 4, 8:00 A.M., both flies were found dormant in the cups. Upon being dissected both were found to be negative for oöcysts and sporozoites. In fly 25, however, some observations were made on an oökinete which yielded a valuable record. An account of this appears on page 00 in connection with the discussion of the cycle in the fly.

Experiment 11, September 21. Fly 26 was taken on infected quail 87, shot near Grizzly Peak. It was placed overnight on quail 285, an infected young bird that had just been trapped at the same place.

September 22, 8:00 A.M., the fly was removed from the bird, and dissected. Under the oil immersion, four small oöcysts could be seen on the outer wall of the mid-gut. The digestive tract itself contained only digested blood. There were no mature oöcysts or sporozoites. A permanent mount was not made of the material.

Results positive. This was the first direct evidence that the oöcysts of *Haemoproteus lophortyx* develop in *Lynchia hirsuta.*

Experiment 12, September 27. Flies 27 and 28 were taken on infected quail 91, near Mount Diablo. Both flies were dissected. One of them was not infected. The other contained four large oöcysts which ruptured immediately under the cover slip. Among the ruptured oöcysts, living, actively motile sporozoites could be seen in profusion under the oil immersion lens. This material was fixed and stained, all operations being carried out without removing the cover slip. The finished preparation stained with iron-haematoxylin was used for the photomicrograph, figure 1, and the drawing, figure 2, of plate 1.

Experiment 13, October 5. Flies 30, 31, and 32 were taken on quail 93, an infected bird shot near Mount Diablo. Fly 30 contained eleven discernible oöcysts, four of which ruptured immediately when the material was compressed under the cover slip. Under the oil immersion lens living sporozoites could be seen in abundance. The cover slip was removed and washed gently with a jet of normal saline from a capillary pipette. These washings were injected intramuscularly into quail C1, the bird receiving about ½ cc. of the fluid. The salivary glands of the insect and the remaining contents and residue of the oöcysts were chopped up on the slide in normal saline and injected into the wing vein of quail C2.

Results. Quail C1 remained negative for *Haemoproteus.* Bird C2 gave a positive record of successful transmission. The case history follows:

Bird C2, up to 26th day negative.

27th day, a few earliest stages of gametocytes found in the blood, not recognized at the time as *Haemoproteus.*

30th day, positive identification of early gametocytes in the blood. About twenty-five per blood slide could be found.

34th day, gametocytes still few in number but some of them had reached the pigment producing stage.

35th day, gametocytes not increasing in number.

36th day, gametocytes fewer in number, but increased in size, some of them half as long as the nucleus of the blood cell.

38th day, no gametocytes could be found that contained pigment. There seemed to be a sharp drop in number also.

42nd day, gametocytes entirely disappeared from the blood.

44th to 52nd days, examinations continued to give negative results.

64th day, blood negative.

Examination made at intervals showed no evidence of recurrence of gametocytes in the blood. The bird was killed on the 146th day. Examination showed no *Haemoproteus* lesions. There were no schizonts or evidence of schizogony in the lungs and spleen. Whether the limited number of sporozoites introduced into the blood of the bird, or whether an active immunity of some kind prevented the infection from becoming more permanently fixed in this bird is not known. The bird at no time showed symptoms of disease.

Under natural field conditions it is probable that a covey of quail harbor at least one fly per bird. The habits of quail are such that flies may move readily from bird to bird. It is also probable that the flies live several days at least and that they feed daily. Under such conditions a quail would be far more liable to incur infection than under the experimental conditions described above, and made necessary by the scarcity of flies.

Even though the one case of positive transmission failed to establish a permanent infection in the bird, it is believed to be conclusive when analyzed from the standpoint of the other findings. These findings were briefly: the discovery of oöcysts and sporozoites in flies taken on infected quail, and the fact that two lots of young quail reared in such a way as to be protected from hippoboscids remained negative for *Haemoproteus.*

TISSUE TRANSPLANTING EXPERIMENTS[1]

In order to ascertain whether a *Haemoproteus* infection can be transmitted by transplanting living tissue from one bird to another, some tissue-grafting experiments were carried out. Chickens were used at first instead of quail as the number of quail available for such experiments was limited, and it was not known how high the mortality might be. It was also thought that the use of chickens might serve the additional purpose of testing host-parasite specificity. The experiments were as follows:

Experiment 1, September 6. Quail 31, a bird with an old chronic infection, was killed and one lung divided into three portions. One of these pieces was introduced subcutaneously into each of three ninety-day-old chickens. Surgical precautions were taken in performing the slight operations, which were done without anesthesia. The lung tissue had not been allowed to cool before it was introduced into the chickens. The wounds healed quickly with no bacterial infection developing. All the birds remained negative for *Haemoproteus.*

Experiment 2, September 6. Chicken 4 was etherized and an opening made into its body cavity in much the same manner as for caponizing a fowl. Half of the remaining lung of the quail used in the preceding experiment was introduced into the body cavity of this bird. Aside from the ether sickness, the chicken showed no unfavorable symptoms. Recovery was good. No bacterial infection set in. This chicken, likewise, remained negative for *Haemoproteus.*

Experiment 3, September 6. The remaining portion of the lung of quail 31 was introduced subcutaneously into quail B1, in the same manner as had been done in the case of the chickens. The bird recovered quickly, the wound being entirely healed in three days. This quail remained persistently negative for *Haemoproteus.*

Experiment 4, November 12. Quail 285, a six-months bird with an acute active *Haemoproteus* infection, was etherized and the lung tissue from it transplanted into the body cavity of quail D. Ether-anesthesia was used and proved fatal to the bird that received the tissue transplant.

Experiment 5, November 12. Quail D2 was used in a second experiment duplicating experiment 4. In this case care was taken to anesthetize the bird more lightly. An entire lung from quail 285 was introduced into the body cavity of this bird. Recovery was excellent. The case history follows:

4th day, wound entirely healed, no bacterial infection.

8th day, first blood sample taken, no gametocytes in the blood. Blood count 3,800,000.

12th day, many erythrocytes had a ragged appearance. Blood count 3,600,000.

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[1] Suggested by Dr. K. F. Meyer.

13th day, bird droopy, destruction of erythrocytes more pronounced.

14th day, bird sick, inactive. Blood count 3,400,000.

15th day, bird weak, seemingly at point of death.

16th day, merozoites of *Haemoproteus* had appeared in great numbers in the circulating blood. Coincident with this appearance of merozoites the bird seemed to be improving.

17th day, many young gametocytes seen in the erythrocytes.

18th day, heavy *Haemoproteus* infection. Six per cent of the blood cells infected. The bird, however, was in better condition than it had been from the 14th to the 17th days.

20th day, the gametocytes had reached the pigment producing stage.

22d day, no further change.

24th day, the gametocytes were becoming less numerous, about 4 per cent of the cells infected.

30th day, only an occasional gametocyte could be found in the blood. None of them had reached maturity.

40th day, blood negative for gametocytes.

The blood of this bird continued to be negative but the bird was thin and in poor condition. On December 31 it was removed from the inside cage and placed outdoors with some other quail in a pen. It died January 13, presumably as a result of exposure following cold wet weather. Autopsy showed no trace of the implanted tissues. The spleen and lungs were somewhat pigmented but no schizonts were found.

Experiment 6, December 7. Quail 33, a bird with a chronic *Haemoproteus* infection, was killed by bleeding, and a portion of the lung introduced into the body cavity of quail D3. No anesthesia was used for performing the operation. The bird recovered quickly, the wound being entirely healed in four days. This bird remained active and healthy and its blood was persistently negative for *Haemoproteus* until March, when it was used in experiments with quail disease other than *Haemoproteus.*

Experiment 7, December 7. This was a duplicate of the preceding experiment, quail D4 receiving a lung transplant from quail 33. As in the case of quail D3, recovery was excellent and the bird remained negative for *Haemoproteus* until March, when it also was used for other experimental work.

From the above experiments it will be seen that a *Haemoproteus* infection can be transmitted by tissue transplants from a bird with an active case of the infection. This is probably due to the fact that schizogony is taking place in the tissue at the time of transplanting and that new host cells become infected readily. The failure to transmit the infection by transplanting tissue from a chronic case can be explained upon the basis of the absence or scarcity of schizonts in such cases. Histological studies of the tissues of acute and of chronic cases confirm this supposition. What part immunity plays in the transmission results is not known. Quail D2 may have been able to throw off the infection owing to an immunity which may have developed because of the severity of the initial attack. Should an attack as severe as this develop under natural field conditions as a result of fly transmission, it is doubtful whether a bird could survive it. In fact, one fatal field case observed, that of quail 46, was much like this experimental case as to the behavior of the bird and the nature of the infection. This bird (no. 46) when captured June 8 was in a greatly weakened condition. Just before its death on the following day, large numbers of merozoites appeared in its blood and it exhibited disease symptoms similar to those shown by quail D2 at the height of its sickness.

LIFE-HISTORY OF *Haemoproteus lophortyx*

SEXUAL CYCLE

Formation of microgametes—

The terms "flagellating body," "exflagellation," and "flagellation process" are firmly established in the literature dealing with malaria. To substitute other expressions would remove associations linked with the names of the French army surgeon, Laveran, the brilliant British army officer, Major Ross, and the keen young American pathologist, MacCallum. But since the process is truly one of gamete formation, it will be referred to as such in this paper.

It is well known that when blood containing "ripe" male gametocytes is placed between a slide and a cover slip at room temperature, male gametes are produced. The "flagellating process," or more specifically gametogenesis, as first correctly interpreted by MacCallum (1897), has been described in various ways. The commonly accepted description that has found its way into textbooks runs something like this: the male gametocyte comes out of the blood cell, rounds up, and throws out "flagella." In the meantime, chromatin material from the nucleus moves out into these protoplasmic filaments. They lash about for a few minutes, break away, and become free microgametes.

Various figures of the process, of the free male gametes, and of the residual mass of the gametocyte, have been published. Schaudinn (1904) shows the nucleus of the male gametocyte in the blood cell divided up into six spherical masses, before the gametocyte has even begun to round up. It is curious how various modifications of this figure still persist in the literature. Anschütz (1909) describes and figures the process as he observed it in *Haemoproteus orizivorae.* He shows microgametes in the process of emerging in the conventional manner. Brug (1916) has a different interpretation as to how the microgametes are formed and become free. He worked with *Proteosoma precox.* His interpretation of the process was that the nuclear material of the gametocyte spread out in peripheral or concentric bands, and split off into segments to form the nuclei of the microgametes. Wasielewski and Wülker (1918) describe the process as occurring in the *Haemoproteus* of the Türmfalken and publish figures of the free microgametes and of the residual mass of the gametocyte after the gametes have been formed. They show material that takes the chromatin stain in this residual mass.

Good descriptions of behavior during the process of microgamete formation based upon the study of living material are wanting. Similarly, the behavior of the nucleus of the male gametocyte immediately preceding the emergence of the microgametes has apparently not been studied thoroughly. Revised figures are also needed of the microgametes themselves and of the remaining substance of the gametocyte after gamete formation is completed.

In some recent work with *Plasmodium kochi* of the monkey, Anderson and Cowdry (1928) describe the process of the formation and liberation of the microgametes. They made use of the dark field as well as the oil immersion lens for the study of living material. These writers conclude that the male gametes are fully formed inside the gametocyte and that they escape by emerging with either the anterior or the posterior end foremost.

The work of these writers with the malarial parasite of the monkey and the present work with *Haemoproteus lophortyx* is essentially in agreement as to the behavior of the male gametocyte and the microgametes during and after gametogenesis.

The following description of the process as it occurs in *H. lophortyx* is based upon observations extending over several months and has involved the study of gametocytes from the blood of numerous infected quail. The first outstanding observation that one makes is that the gametocytes must be ready to produce gametes or they will not do so. Mere chilling will not induce the process. Blood full of gametocytes from a heavily infected bird may be placed on a slide with very little activity, if any, taking place. A few hours later, blood from the same host may show gamete formation in profusion in every field under the oil immersion. Just what the stimulus to gamete formation is, was not determined. It does not seem to be temperature or any dilution factor. It was observed in whole blood, in blood diluted with normal saline solution, and in blood diluted with the juices of the transmitting host.

The activity observed is as follows: Within one to eight minutes, but usually nearer the lower figure, after the drop of blood is drawn and the slide made up, the male gametocyte rounds up rapidly, the process requiring from ten seconds to two minutes. It is possible that as the gametocyte grows within the blood cell, its cell membrane becomes attached to the cell membrane of the erythrocyte. As a result of the rounding process, the nucleus of the blood cell may then be extruded. At any rate, this nucleus becomes freed from the blood cell and remains adherent to the spherical gametocyte. Meanwhile the pigment granules begin to dance about, at first mildly, then more violently. A sol state of the cytoplasm of the gametocyte appears just inside the periphery. It is in this region that the pigment granules dance about. Some tremendous activity at the center of the mass keeps them away from that area. This center of activity becomes decentralized or broken up into two or more centers. Presently the movement of the granules indicates that serpent-like organisms are milling about within the gametocyte. The gametocyte may twist and turn and contract and expand. Then suddenly the male gametes emerge fully formed. The activity of the gametocyte previous to the appearance of the gametes is strikingly like that of the living cells in tissue culture as shown by the Canti (1928) motion pictures.

This activity of the gametocyte is intensified by that of the adhering microgametes which is sufficient to move the gametocyte about within a circumscribed area. Such activity of the gametes may pull or propel, rotate, or tilt the gametocyte from side to side. Microgametes are extremely active, slender, vermicular organisms. They vary in length from three-fourths to one and one-half times the length of an erythrocyte. In fixed, stained preparations their average length is 13.5 microns. There is definite polarity, the long slender nucleus being anterior. Under the dark field, a less refractive conical point is discernible anterior to the nucleus. This conical point is provided with a minute refractive granule. Within the nucleus is a much larger refractive granule, probably a karyosome. It is the appearance of this structure in the living state that enables the observer to keep the gamete oriented. In stained preparations this granule is more compact than the rest of the nucleus. Compare figures 9 and 29, plates 1 and 2.

Under the dark field, the microgametes can sometimes be seen inside of the gametocyte, with their bodies bent into arches (see fig. 8, pl. 1). The pushing of the ends and the centers of these arches against the limiting membrane of the gametocyte accounts for the elbowing movements of the gametocyte so often seen. When the arches of several of these microgametes lie alongside each other just inside the periphery of the gametocyte, they exert enough force to rupture the membrane.

The method of their exit cannot be described in general terms applicable to all cases. Two have been observed to emerge at the same time and place, "head first" and to free themselves in ten seconds. Three have been observed shooting out "tail first" and thrashing about for approximately four minutes before the first one became free. On one occasion a gametocyte was observed to rupture at one place and two rounded masses of cytoplasm each containing a gamete were extruded. These gametes writhed about, both of them escaping head first in about two minutes. A few seconds later a third cytoplasmic sphere containing a gamete came into view through a rupture. In two minutes more a fourth sphere arrived, much smaller than the preceding three. Gamete 3 straightened out tail first almost immediately, but it did not become entirely free until six minutes later. There was only feeble activity in sphere 4 as if this attempt to produce a gamete were abortive. Part of the pigment granules meanwhile had escaped from the gametocyte which had given rise to the gametes, and there was no further activity of the remaining granules within the residual mass.

The activity is so violent that it is almost impossible to count the microgametes as they emerge. On numerous occasions, it seemed certain that only four escaped. There is evidence that sometimes as many as eight are formed. When a gamete comes out head first, this fact can be ascertained, under the dark field, by the appearance of the refractive granule near the anterior end of the gamete. The movement is also different but hard to describe. It is as if a serpent tied by its tail were making vigorous efforts to escape. On the other hand, when a microgamete emerges tail first, the movement observed before it frees itself is more vibratile. On some occasions it appears as if a microgamete partly emerges tail first and is then drawn back into the mass of the gametocyte. When several gametes have been shot out and are still adhering to the residual mass, their movement is not synchronized and regular but more of an individual matter. The gametes are not all the same size. The most common method of emergence is for four gametes to escape through a common rupture. Long after all the gametes have apparently escaped, even up to fifty-five minutes, remaining pigment granules have been observed to continue what appear to be brownian movements within the residual mass of the gametocyte. Also, similar movements of pigment granules have been seen to take place for about an hour in gametocytes from which no gametes whatsoever emerged.

Once free in the plasma, microgametes move about with a serpentine or an oscillating motion. They are extremely powerful and can thrust red blood cells aside. They have also been observed to attach themselves by the anterior end to red blood cells and shake the cells vigorously before they succeed in becoming free. On rare occasions they have been seen to enter red blood cells. One feature of the behavior of a microgamete which is very common is for the gamete to glide around over the surface of a red blood cell or leucocyte, with the anterior and posterior fourths of its body in flat contact with the periphery of the blood cell and the central portion of its mass arched out.

Microgametes may exhibit polymorphism as regards alternate swelling and contracting of the anterior portion. The nucleus is apparently shifted from place to place by a euglenoid motion of the anterior portion of the gamete. In order to obtain good fixed preparations of the microgametes, it is necessary to prepare the material immediately after free microgametes are available in the field; otherwise they may present a distorted appearance. It is probable that the long slender forms apparently devoid of a nucleus, sometimes seen in fixed preparations, are only the tails of microgametes whose nuclei have become trapped in the fertilization membrane of the zygote. Such tails have been observed to break off in the living preparations.

A significant fact learned from the study of slides made up at intervals of two minutes after a drop of blood is drawn and covered by a cover slip, is that microgametes are formed as a result of regular divisions of the nucleus of the gametocyte (figs. 19 to 23, pl. 1). When a male gametocyte is "ripe," its nucleus is diffuse and spread out over at least three-fourths of the volume of the cell. When the cell has almost rounded up preparatory to gamete formation, this nucleus looks not unlike the coarse spireme stage of the nucleus of any cell undergoing mitosis. The nucleus contracts rapidly and then appears as a small central mass surrounded by an enormous area of clear cytoplasm. This nucleus divides into two parts, which in turn divide again, making four. Cells have been seen showing all numbers of nuclei from one to six. Neither in the stained preparations nor in optical properties seen in the living material is there any evidence that each nucleus is surrounded by a mass of cytoplasm cut off from the rest by a cytoplasmic membrane. The special case of gamete liberation previously described, however, offers strong evidence that this is the case. It would also explain why a microgamete is retarded in emerging, by the limiting mass of cytoplasm that clings to it. There is also additional evidence for this assumption in the optical appearance of the microgametes as they mill around inside the gametocyte.

Microgametes are short-lived. In from eight to twenty minutes they become less active, swell, or become cytolyzed and beaded and then disintegrate. If gametogenesis has been completed, there is no chromatic substance left in the residual mass. The chromatin substance often found in the residual mass after gametocytes have been liberated on a slide is believed to represent the remains of aborted gametes.

Formation of macrogametes—

During the time in which male gametocytes are emerging from the blood cells and rounding up preparatory to forming the microgametes, the female gametocytes are likewise undergoing change. As described on page 9, the female gametocytes are distinguished from those of the other sex by their smaller, more compact nuclei and by differences in the number and arrangement of the pigment granules. In the living condition, the cytoplasm is more dense and opaque than it is in the male gametocytes. Stained with Giemsa, it is much darker blue.

The rounding up movement of the female gametocyte as it emerges from the blood cell is more sluggish than that of the male, requiring from three to eight minutes. Stained preparations made from material fixed immediately after this process is completed, show that in many cases the nucleus has already migrated to the periphery of the cell preparatory to throwing off the first polar cell. The formation of the first polar cell requires from two to four minutes. It usually appears on the side of the gametocyte opposite the quiescent nucleus of the destroyed blood cell. It is about one-tenth the diameter of the macrogamete. During its formation there is slight movement of the pigment granules, but no general agitation of the cytoplasm comparable in intensity to that seen in microgamete formation.

In from six to ten minutes after the appearance of the first polar cell a second one may be given off by the gametocyte. It may be adjacent to the first or at some distance away along the periphery of the cell. It is about the size of the first, but its formation is more rapid. As before, slight movements of the pigment granules within the cell may continue. Meanwhile the body of the cell has become a macrogamete. No further change takes place unless fertilization occurs. The second polar cell has sometimes been observed to be thrown off after the microgamete has entered the macrogamete in the process of fertilization. The polar cells degenerate quickly and become diffuse. When fixed just at the right time, they take the nuclear stain. This, it would seem, is evidence that chromatin material is present. Whether either one of the cell divisions which result in the formation of polar cells is a reduction division is not known, for no chromosomes have been distinguished in the chromatin of the nucleus.

Fertilization—

Fertilization of a macrogamete by a microgamete was first discovered by MacCallum (1897). In describing the process briefly, he refers to the microgamete as concentrating its protoplasm at the anterior end and then dashing into the macrogamete. He noted also the agitation of the cytoplasm as evidenced by the movement of the pigment granules immediately after the microgamete had gained entrance.

This phenomenon has been studied many times and the details of the process worked out. The actual penetration of the microgamete into the large passive macrogamete takes place in about fifteen seconds. In order to observe all the events in sequence, it is necessary to focus on a female gametocyte immediately after the blood slide is prepared. The gametocyte chosen may not be ready to undergo maturation, or in case it is ready, the microgamete may not be available for fertilizing it. In this event, another drop of blood must be prepared and another trial made.

The following account copied from the laboratory notes is a typical example of just what occurs:

A.M.
9:35. Drop of blood drawn and slide prepared.
9:39. Female gametocyte beginning to change form.
9:42. Has completely rounded up, nucleus of blood cell is extruded.
9:46. First polar cell beginning to appear.
9:51. Polar cell complete.

A.M.

9:52. Seven microgametes have appeared from one side of the field of the microscope. They are all within a distance of three long diameters of an erythrocyte from the macrogamete.

9:53. The anterior end of the first microgamete has made contact with the macrogamete; it becomes attached instantly; the serpentine movements slow down; the microgamete penetrates about one-fourth of its length; a swelling of the microgamete nucleus appears at the point of contact as if the nuclear material were streaming forward; the tail of the gametocyte bows around and touches the macrogamete at another place; it swings back; three feeble undulating movements of the microgamete occur; its entire body disappears within the macrogamete. Time from first contact to disappearance of microgamete, fifteen seconds.

9:53′ 15″. The pigment granules which have been moving ever so slightly a few at a time, and at various places in the large cell since 9:42, are now in active motion as if the microgamete inside were moving about. Some of this movement is due to the activity of two more microgametes which are now attached to the macrogamete and thrashing about, unable to gain entrance.

9:55. The movement of the pigment grains is slowing down; it is restricted; the amplitude is smaller; the pigment grains are being concentrated into vacuoles.

9:56. A second polar cell is forming; it is the same size as the first and is adjacent to the former but is being formed from the macrogamete and not by division of the first polar cell.

9:57. A diffuseness has spread over the macrogamete. The pigment granules are quiet. They do not move when the zygote is disturbed by the microgametes which are still attached to it.

9:58. The first polar cell divides quickly, giving rise to two smaller daughter cells. The diffuse appearance of the zygote, the refraction of light from its surface, and the enmeshing of portions of some of the microgametes indicate that a fertilization membrane was formed immediately after fertilization.

10:01. One of the attached microgametes pulls loose and swims away; its movements are not normal; part of its anterior end is missing.

10:02. The second attached microgamete breaks away, leaving its nucleus imbedded in the zygote.

10:10. All the polar cells are becoming diffuse.

10:20. Only an indefinite mass remains where the polar cells were. The pigment granules are quiet and all of them are peripheral in position, appearing in three different focal planes.

10:27. An almost imperceptible elongation of the zygote indicates that the oökinete is beginning to form.

10:30. No further observations made.

The entrance of more than one microgamete has never been observed. The study of living material indicates that the complete penetration of one microgamete effectively blocks the entrance of any others. On the other hand, the study of both living and preserved material indicates that it is common for several microgametes to become trapped in the fertilization membrane, and to remain attached or break away, leaving part of their protoplasm. A living zygote with several microgametes adhering to it and thrashing about vigorously might easily be mistaken for a "flagellating" male gametocyte. In stained preparations, this same situation might easily lead the observer astray, causing him to think that the usual method of emergence of the microgamete is tail first, with the nucleus toward the proximal end.

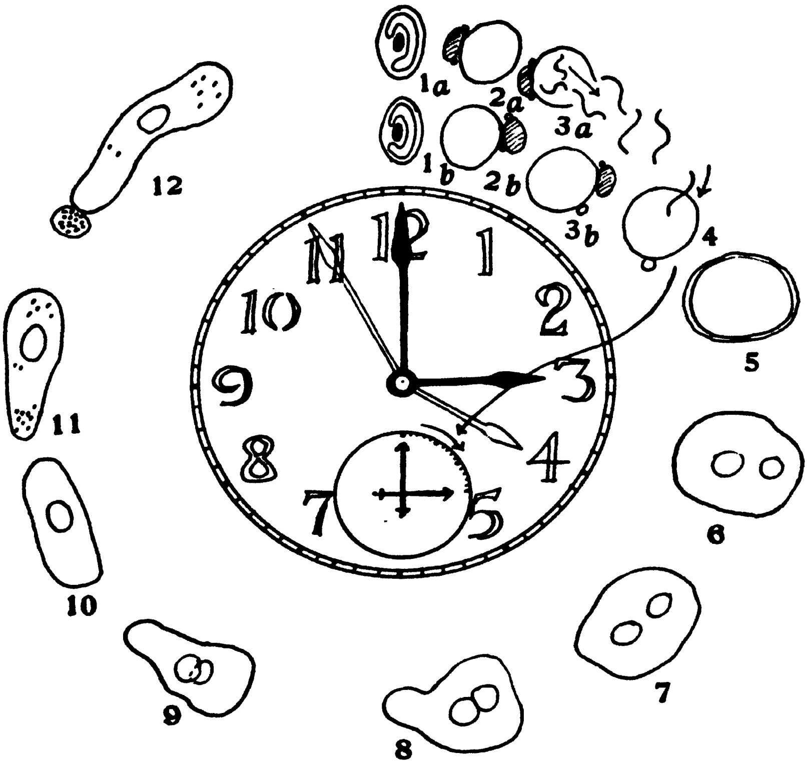

Fig. C. Diagram showing the time element involved in the events of gametogenesis, fertilization, and oökinete formation on the microscope slide at room temperature. *1a*, male gametocyte in blood cell; *1b*, female gametocyte in blood cell; *2a* and *2b*, gametocytes out of blood cell and rounded up; *3a*, gametogenesis, male gametes formed and liberated; *3b*, gametogenesis, female gamete formed; *4*, fertilization; *5*, fertilization membrane formed; *6–7*, resting zygote; *8–10*, elongating oökinete; *11–21*, motile oökinete.

ASEXUAL CYCLE

Development of the oökinete and sporogony—

Both the Sergents (1906) and Aragao (1908) observed the oökinetes of *Haemoproteus columbae*, and Adie (1915 and 1921) studied them extensively in connection with sporogony in *Lynchia maura*. She stated that the oökinetes developed in the stomach of a fly a few hours after an infective feeding.

The writer has on numerous occasions watched the development of oökinetes of *Haemoproteus lophortyx* under the oil immersion lens on a microscope slide at room temperature. In general, the zygote rests for about twenty minutes before it begins to elongate. The shortest elapsing time after fertilization in which a zygote was seen to begin the process was fourteen minutes. The movement is ameboid and almost imperceptible. In about twenty minutes the elongation is accomplished and the oökinete begins to stream slowly across the field. In the meantime, while elongation is taking place, the majority of the pigment granules are left toward the posterior end as the protoplasm streams anteriorly. As the substance becomes increasingly transparent, the male and female pronuclei can be seen approaching each other. Sometimes a blob of cytoplasm containing pigment is pinched off posteriorly. This occurrence is more frequent in blood diluted with normal saline than in undiluted blood. In stained preparations of material fixed fifty-five minutes after the drop of blood was drawn, many oökinetes can be seen. In some of them the pronuclei are separate and distinct, while in others they are almost fused.

On one occasion the penetration of an oökinete into the wall of the gut was observed. The fly which furnished the material had been captured on an infected bird about four o'clock in the afternoon. It was placed in a biting cup on another infected bird and left overnight. The next morning the fly was dormant and apparently dead. Upon being dissected out, however, the intestinal tract of the fly was found to be alive, exhibiting peristaltic movements. When flattened under the cover slip and examined with the oil immersion lens, the material was beautifully transparent. Among whole and partly digested red blood cells were a number of rounded gametocytes, whose sex could not be determined, together with three oökinetes. Two of these were inactive but the third was apparently just attaching itself to the inner wall of the gut. To permit continued observation the edge of the cover slip was then sealed. Ever so slowly, the forward end of the oökinete penetrated the wall, whether intercellularly or intracellularly could not be determined. It is interesting to note that the narrower, more transparent end of the oökinete entered the wall of the gut first as distinguished from the entrance of the broader end as shown in the Perrier and Cépède (not dated) chart illustrating the life-cycle of the malaria parasite. In about fifty minutes the posterior end of the oökinete had disappeared from the lumen of the gut. Seventy minutes later a tiny swelling appeared just beneath the outer membrane of the gut, and because of its greater opacity was believed to represent the earliest formation of an oöcyst. No further change occurred, and six hours later all the tissues were dead and transparent.

When Adie worked out the sporogony of *Haemoproteus columbae*, she found that the oöcysts could be recognized on the wall of the gut in four days after an infective feed on the part of *Lynchia maura*. Oöcysts reached maturity in about ten or twelve days whereupon they burst, liberating thousands of sporozoites. After the thin-walled oöcysts had burst, there was a residual core of protoplasm remaining. She found that the sporozoites penetrated numerous tissues of the host, and in particular the salivary glands, engorging these tubular organs. The sporozoites measured up to 10 microns long. Some of the oöcysts attained a diameter of 36 microns.

The present observations made on *Lynchia hirsuta* are in agreement with Adie's findings. Adie's flies were, however, more heavily infected than the ones used in this study. The greatest number of oöcysts found in any one specimen of *Lynchia hirsuta* was eleven, only four of which were mature. These mature oöcysts burst before they could be measured. The sporozoites moved with a vibratile motion. In the living condition, they seemed to be larger and more vermicular than those figured by Adie but the stained preparations are similar. The nucleus of the sporozoite of *H. lophortyx* is more elongated than that of *H. columbae* (see figs. 11 and 12, pl. 1).

Schizogony—

When the oöcysts on the wall of the gut of the fly are mature, they rupture, discharging great numbers of sporozoites into the haemocoele of the insect. These minute, slender, vibratile bodies are capable of active movement. They penetrate through the tissues of the host, enter the salivary glands, and fill the central tubules of these organs. A fly with its salivary glands thus infected is infective to the vertebrate host upon which the fly feeds, the sporozoites being discharged through the ducts of the salivary glands. From the appearance of the longitudinal bands of muscle encircling these glands, it is probable that they function in causing the sporozoites to be discharged. The exact course of the sporozoites in the blood stream of the bird has not been determined. Neither is it known just how soon schizogony, the next step in the life-history of the parasite, begins.

Schizogony was described by Aragao (1908) from the lungs of pigeons infected with *Haemoproteus columbae.* MacCallum (1897) had observed the ovoid bodies in the liver of infected birds, but he did not recognize them as being a stage in the life-history of the parasite.

According to Aragao, a sporozoite upon entering an endothelial cell in the capillaries of the lungs undergoes growth accompanied by multiple division of the nucleus. The cytoplasm of this enlarged cell, or schizont as it is now called, becomes divided into cytomeres. The nucleus of each cytomere in turn undergoes multiple division. Schizogony is believed to be repeated a number of times before merozoites capable of infecting blood cells and becoming gametocytes are produced. Previous to their liberation, these merozoites can be found in the endothelial cells which have become free in the capillaries.

Wenyon (1926) found that the schizonts of *Haemoproteus columbae* in the pigeon do not necessarily produce cytomeres before giving rise to merozoites. He speaks of the schizont as being a large sausage-shaped body with innumerable nuclei. He found that schizonts were lodged in the endothelial cells of the capillaries and that they may send out branches along the bifurcations of the capillaries, becoming triradiate or multiradiate. Various observers have noted that schizonts may be found in the lungs, liver, spleen, and kidneys.

In the course of the present work, schizonts have been found in the lungs, liver, and spleen. The findings agree with those of Wenyon as to the sausage-shaped appearance. This was especially noticeable in the spleen. Slight branching could be noticed in thick sections of spleen running from 10 to 15 microns. Just where and how merozoites enter the red blood cells is not known. They have been observed only rarely in the circulating blood. If they were commonly present there in large numbers, transmission by direct blood inoculation should be easily accomplished. On one occasion one-tenth of a cc. of blood containing free merozoites from a heavily infected quail was injected subcutaneously into each of two susceptible quail. The blood was diluted ten times with normal salt solution. Computations made at the time of the inoculations indicated that each quail received 300,000 free merozoites, yet neither bird became infected.

PATHOLOGY: DISEASE SYMPTOMS IN INFECTED BIIRDS

From the early literature it is not clear whether the Haemocytozoa to which reference is made belonged to the genus *Haemoproteus* or the genus *Proteosoma* or both. Danilewsky (1889) describes macrophages which contain masses of pigment. He also mentions shrunken red corpuscles and the remains of parasites. He describes the blackened spleen and states that pigmented cells are enclosed in large macrophages in this organ. He refers to the changes in the bone marrow as being similar to those observed in the spleen.

Kruse (1890) found pigment enclosed in large clear cells in the capillaries of the lungs, spleen, liver, and bone marrow. He believed these conditions to be due to the acuteness of the infection, arguing that, in all probability, the pigment would be contained in fixed cells if the infection were chronic.

Labbé (1894) gives a brief general discussion of gross pathological changes in birds infected with the parasites. His comments are not essentially different from those of Danilewsky.

MacCallum (1898) made gross and microscopic studies of material furnished by autopsies performed on about 125 birds belonging to various species. The *Haemoproteus* infecting these birds had been recognized by Opie (1898) as being of five different kinds. MacCallum indicated in most cases whether the lesions which he discussed were caused by *Proteosoma* or *Haemoproteus*. He recognized the enlarged, blackened spleen as a characteristic lesion in *Passer domesticus*, *Corvus americanus*, and *Agelaius phoeniceus*. He also noted that the liver was large and pigmented. He found areas of focal necrosis containing central hyaline deposits in the liver. Various regular symmetrical groups of ovoid bodies were also seen in the liver. In one case he found such ovoid bodies associated with a focal necrosis. In some but not in all cases MacCallum found pigmentation in the bone marrow. In the light of later investigations it is clear that the ovoid bodies seen by him were stages of schizogony of the parasites. MacCallum was unable to verify Kruse's findings as to the pigmentation of the lungs. He also was not impressed with the intensity of pigmentation in the bone marrow.

The present investigations dealing with *Haemoproteus lophortyx* support MacCallum's findings as to the pigmentation of the spleen. The pigment deposits in the lungs are variable. Some infected birds show scarcely any whereas others have enormous amounts of pigment in these organs (fig. 6, pl. 1). The present records are also in agreement with MacCallum's observations that pigment was not especially noticeable in the bone marrow.

With regard to other organs, pigment deposits have not been observed in the brain, but enormous amounts are present in the testes (fig. 5, pl. 1). Since pigment deposits have also been observed in the non-breeding season in the testes of quail which were believed to be free from *Haemoproteus,* such findings in the parasitized birds may not be significant.

As to the condition of the liver, there seems to be little difference between the livers of normal birds and of those harboring chronic infections. The general appearance is similar and there is little difference in microscopic sections. In the few fatal cases that have been examined, there is an excessive amount of pigment in this organ.

Of the numerous birds shot in the field, no special examination was made of the livers for the comparison of infected and uninfected birds. When the experimental work was brought to a close, however, four uninfected quail and four with chronic *Haemoproteus* infections were on hand. These birds were all adult birds that had been kept at the University under similar conditions. They were all killed at the same time with ether, and comparative body weights and liver weights taken. Before being weighed, the livers were ligated and dissected free. The following table gives the data obtained:

INFECTED BIRDS			UNINFECTED BIRDS	
Body weight in grams	Liver weight in grams		Body weight in grams	Liver weight in grams
124.7	3.3		135.2	5.9
114.2	3.3		132.0	5.9
107.0	3.4		152.2	6.2
155.8	6.0		143.0	6.8
125.4	4.0	Average weight	140.6	6.2

The ratio of body weight to liver weight when reduced to per cent gave the following interesting comparisons. The ratio of body weight to liver weight in the infected birds was 100:3.18; in the uninfected birds it was 100:4.37. The average weight of the parasitized birds was 82 per cent that of the normal birds.

The results of this comparison as to the body weights of the birds were not unexpected, for it had been noticed that birds with chronic *Haemoproteus* infections may be lighter in weight than normal birds. This is apparently not applicable to the light chronic cases so often encountered in the field. It rather represents that type of infection which has been characterized as moderate chronic in this paper (see text fig. E, page 39).

The results of the comparative studies of the liver weights were surprising, as it was expected that the livers of infected birds would be found to be at least as large as those of uninfected birds. It is possible that the general unthrifty condition of a bird harboring this type of infection prevents the normal amount of food storage in the liver and reduces the weight of that organ.

Tissue destruction is more apparent in the blood than elsewhere. The percentage of red blood cells parasitized at any time with gametocytes which are readily discernible may reach 10 per cent and even more. As these gametocytes approach full size and maturity, they replace the cytoplasm, consequently destroying the oxygen-carrying capacity of the invaded cells. Not only is the normal functioning of the parasitized cells interfered with, but there is a pronounced reduction in the number of cells, producing an anemia which may be severe. This is especially true of the heavily infected acute cases. It has been observed, however, in moderate chronic cases. There is evidence also that the blood cells of a heavily infected bird may be destroyed when there appear to be no gametocytes in them. The normal range of blood counts in healthy quail is from 3,800,000 to 4,200,000. In parasitized birds, it has been observed to fall as low as 2,800,000.

The destruction of blood cells can be strikingly shown by staining fresh blood with dilute neutral red. In normal erythrocytes the segregation apparatus takes up the stain beautifully. As the gametocytes grow, this structure is destroyed until in cells containing full-grown gametocytes no vestige of it remains.

The weakening effect of the parasite on the cytoplasmic membrane can be shown by centrifuging fresh diluted blood for a few minutes at high speed. Such centrifuging will break down the parasitized cells more quickly than the normal cells.

Hypertrophy of infected cells is common but not universal. It seems unreasonable to assume that this slight increase in size of the red cells might clog the small capillaries and thus interfere with circulation but such seems to be the case. There are often apparently more mature gametocytes proportionately in smear preparations made from the lungs than in films made from blood drawn from the wing vein. Some observations made on infected blood as it clots under the cover slip throw light on the problem. As the blood cells stream along in little currents of plasma among the network of fibrin, it may be noticed that cells containing mature gametocytes are not flexible like uninfected cells. They cannot bend and submit to compression while going through a small space, and tend to accumulate in narrow or angular passages. It may be the rigidity of the parasitized cells rather than their larger size which accounts for their concentration in the capillaries of the lungs.

While the red blood cells are the ones normally parasitized, all kinds of leucocytes have been found to be infected. The percentage of such cells infected, however, is small, and this type of parasitism is probably incidental. In only a few cases have mature female gametocytes been seen to emerge from eosinophiles and round up as if forming normal gametes.

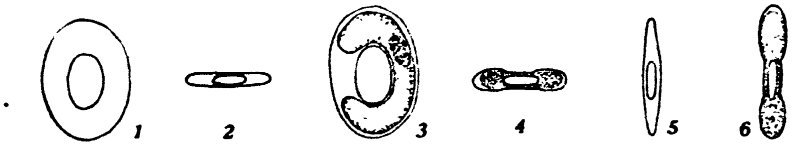

Fig. D. Diagram showing the nature of hypertrophy of the red blood cells caused by the developing gametocytes.

As previously indicated, several investigators have studied the pathological conditions which are revealed by autopsies performed on infected birds. Few, however, have paid any attention to the appearance and behavior of infected birds and to the possibility that disease produced by *Haemoproteus* may be serious and even fatal.

Plimmer (1912) found *Haemoproteus* infections in many cases in birds that died of pneumonia in the London Zoological Gardens.

Wasielewski and Wülker (1918) stated that they knew of a half dozen fatal cases among a thousand birds infected with *Haemoproteus*.

Acton and Knowles (1914) mention a pigeon near death as the result of infection with *Haemoproteus columbae*. Adie (1921) speaks of one of her experimentally infected pigeons as being very ill.

During the present studies, the writer has paid special attention to this phase of the work in behalf of the Division of Fish and Game. Over 300 quail have been actually handled at the State Game Farm, in field and laboratory, and at parks and private aviaries. Field observations have been made on hundreds of other quail, at various localities throughout the year. From ten to twenty infected birds have been under constant observation at the University. Numerous autopsies have been performed. As a result of these observations, four types or stages of *Haemoproteus* disease can be recognized. They are:

1. Mild, chronic. By far the most common, the quail exhibiting no outward symptoms of disease and the presence of gametocytes in the blood being the only indication that the bird is parasitized; extremely persistent, extending throughout the year. Such birds are undoubtedly carriers.
2. Mild, acute. Appears apparently as the result of an initial infection. The bird is restless and off feed for from two to four days, then the infection is either thrown off or settles down to the chronic type.
3. Moderate, chronic. Commonly observed under field conditions. Keeps the bird in an anemic, weakened condition; believed to be the cause contributing to death under such conditions as exposure, or exhaustion following the breeding season.
4. Heavy, acute. Occurs in late spring or early summer, whether as the result of new infective fly bites or the recrudescence of an old infection is not known. The percentage of red blood cells containing gametocytes that have reached the pigment producing stage may be ten and even more. The bird loses flesh rapidly, is unable to fly, refuses food, becomes droopy, and may die. Only four such cases have actually been seen and they are believed to be rare. Occasional reports of heavy losses do come in from the field, however, and it is possible that such losses may be due directly or indirectly to *Haemoproteus* infection.

One ranchman reported that the birds in a covey of quail on his ranch numbering 200 had kept dying off until only 24 remained. He captured two of these and sent them in to the laboratory for examination. Both were infected with *Haemoproteus lophortyx*, one having a light infection and the other a heavy infection. Under observation at the University both cases settled down to the light chronic type.

At another place where a ranchman had reported having found two weak birds which he caught in his hands, sparrow traps were set and four birds were caught. Of these four captured, one, a heavily infected adult, was found dead in the trap. Of the other three, one was normal and two harbored light infections. The dead bird is believed to have been weak and to have died as the result of exertion in trying to escape from the trap.

There has been only one case among the experimental birds where the bird is definitely known to have died from pneumonia following exposure during a cold rain. It was an old chronic case having a moderate infection.

One field case is of special interest in that a sick bird, unable to fly, was caught with the hands and taken to the laboratory, where it died the following day. Autopsy disclosed no lesions other than a heavy infection of *Haemoproteus*.

No acute fatal cases have been observed in young quail, but it is possible that such cases do occur. Fatal endings of chronic cases may possibly result either from relapse, which may be fatal in itself, or from secondary causes which a weakened bird cannot overcome. The following observations made at a private aviary strongly support these conclusions: Thirty quail had died from an acute infection of the digestive tract, the nature of which was not known. The remaining quail in the pen were found to be 100 per cent infected with *Haemoproteus*. As a result of contact with a sick bird from this lot, nine out of ten quail at the University, all of which harbored chronic *Haemoproteus* infections, contracted this acute infection and died. The unknown infection, however, may have been so virulent in itself that even normal birds could not withstand it. Variation in virulence of *Haemoproteus* infections at different times and places, or under different sets of conditions, may be a factor of importance.

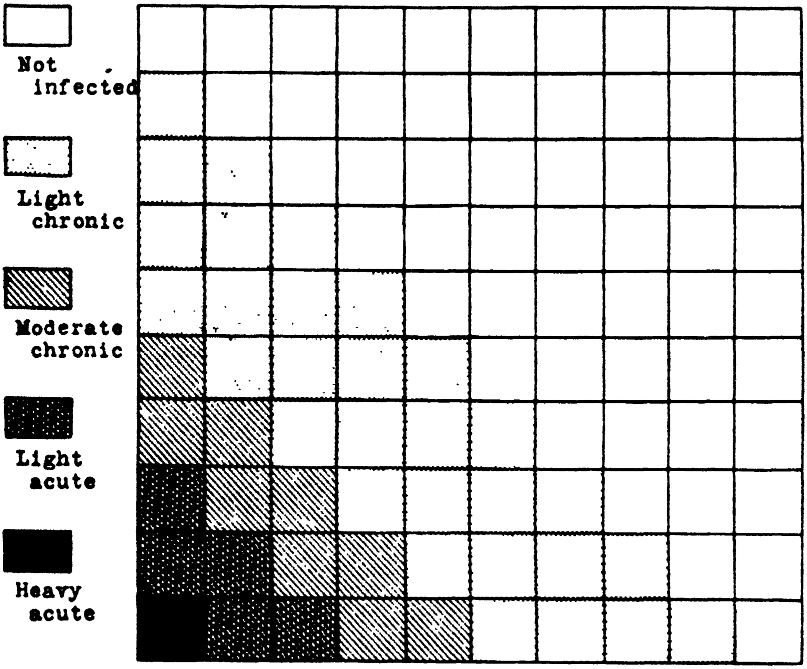

Fig. E. Graph showing the relative percentages of quail harboring the various classes of infection observed. Chart represents 100 birds, sample population.

INFECTION AND IMMUNITY

Since, from what has been described, it is apparent that quail do not become infected unless exposed to the bites of infected flies, it becomes a matter of interest to consider the conditions governing initial infection, the persistence of infections, and possible immunity.

As previously indicated (page 39), infections may vary from extremely mild to fatally acute, and these variations may be due to a number of causes. Among possible causes may be mentioned:

1. Extent of the initial infection, due either to differences in the number of parasitized flies, or possibly to variations in the virulence of the parasites.

2. Natural resistance of varying degree.

Transmission would seem to be facilitated by the observed fact that gametocytes may be found in the blood of quail throughout the year; and that the flies have been taken in nearly all the months of the year. The gregarious habits of the quail would also contribute to the ease of transmission. The actual percentage of infected birds is about forty-five (fig. E). Tending to limit the extent of infection among quail would be the small number of flies observed, the low percentage of infection among them, the light infection of the flies, and their apparent limitation to certain portions of the quail range. Furthermore, the natural resistance of the host is to be considered.

The possibility that *Haemoproteus lophortyx* may yet be found to parasitize birds other than the quail, and that transmitting hosts other than *Lynchia hirsuta* may be found should not be overlooked.

The relative importance of these several factors cannot be definitely determined without further observation and experimentation. Of particular importance would be a complete knowledge of the biology of *Lynchia hirsuta* and any other hippoboscid flies that may be found to be parasitic on the quail.

In the case of parasitism of the pigeon by *Haemoproteus columbae*, it is known that gametocytes may be absent from the blood for months at a time and then reappear. It is further on record that, after having once been infected and having become free from gametocytes, a pigeon is not immune to a new infection.

In quail infected with *Haemoproteus lophortyx*, a few young and developing gametocytes can be found in the blood of old chronic cases at any and all times. The presence of these young forms may be due to any one of the following possibilities:

1. Arrested development of the parasites.
2. New infections from fly bites.
3. Schizogony continuing in the lungs or elsewhere.

If there is continuous turnover in the destruction of old blood cells and the formation of new ones, it seems possible that the first hypothesis is untenable. If it can be proved by experimental means that no new infective fly bites occur, the second hypothesis will likewise not stand, and the third must be accepted as accounting for the condition that has so often been observed.

The continued presence of young gametocytes in the blood of old chronic cases of infected quail which were believed to harbor no flies, furnished statistical evidence that the third hypothesis is the correct one, but it was not accepted until after subjection to rigid experimental test.

On October 7, 1928, three quail having chronic infections were examined carefully and found to be free from flies. They were then placed in a screened cage in the laboratory and kept under observation until January 21, 1929. Young and immature gametocytes could be found in the blood of all the birds throughout the length of the experiment. All of the cases were light infections, with less than 1 per cent of the blood cells parasitized at any one time. On the average only about one in twenty-five parasitized cells contained a young gametocyte.

Contrasted with this is the high percentage of young gametocytes often found in early infections in young birds. In such cases fully 80 per cent of the infected cells may contain young or developing forms. Furthermore it is noticeable that in such infections multiple infections in a single cell are common. On the other hand, it is rare to find multiple infection in the red blood cells of old chronic cases where the number of cells infected with young gametocytes is low. (See fig. F, below.)

The whole problem of immunity among birds to *Haemoproteus* infections is probably in need of serious reinvestigation. Aragao (1908) stated that only in completely healthy birds could an infection be brought about, and that pigeons that had once been infected seemed to be more or less immune to the disease. Adie (1921) believed that her observations indicated that pigeons could be reinfected as many times as infective flies were allowed to feed upon them. She found that young pigeons gradually lost infections which they had developed shortly after leaving the nest. She then allowed infected flies to feed upon the birds from time to time and found fresh crops of young gametocytes appearing in waves, corresponding to the incubation cycle following infective feeds.

In the present studies of *Haemoproteus lophortyx* in the California Valley Quail, two heavily parasitized birds harboring old chronic infections produced young gametocytes in abundance for a period of two weeks during January, presumably without new infective fly bites. Similarly, three birds harboring light chronic infections produced young gametocytes for a period of three months while effectively screened against exposure to hippoboscid flies. There was no evidence in any of these cases that the appearance of young gametocytes was cyclic or in waves. It is not known what caused the reappearance of pronounced numbers of young gametocytes in these old chronic cases, but the possible influence of provocative stimuli should not be overlooked.

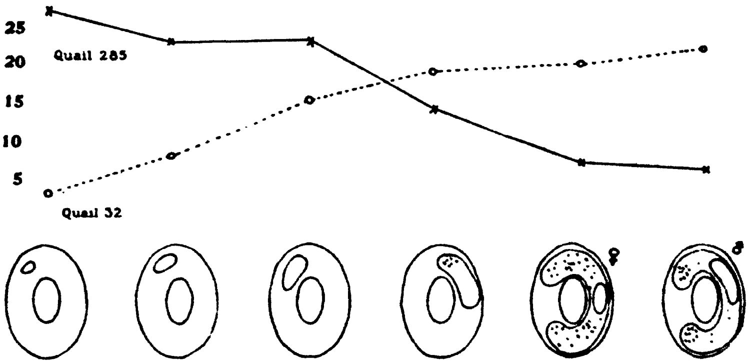

Fig. F. Graph showing comparative numbers of the different stages in the development of gametocytes in quail 385, a young bird with an acute infection, and quail 32, an adult bird with an old chronic infection. Graph represents 100 blood cells. random count from each bird.

The present studies have not dealt with the subject of immunity in an experimental way, and the observations herein listed are only incidental. It does seem, however, that with a disease-producing organism, such as *Haemoproteus lophortyx* evidently is, immunity may be a factor of considerable importance. However, the work of Hegner and MacDougal (1926) and Hegner (1929) indicates that the decline in numbers of parasites in bird malaria caused by *Proteosoma* may be due to interference with sugar metabolism rather than to the development of immunity in the host.

The high incidence of infection among birds in general in certain parts of the Old World, and the low percentage of fatalities suggest a balanced relation between host and parasite.

The highly variable conditions of infection and fatality among the California Valley Quail suggest that such balanced host-parasite relations are not so stable as they are in some of the Old World birds.

SUMMARY AND CONCLUSIONS

1. A species of *Haemoproteus* differing from other described forms has been discovered in the blood of the California Valley Quail and is herewith described as *Haemoproteus lophortyx* sp. nov.

2. Transmission is effected by *Lynchia hirsuta* Ferris, a louse fly of the family Hippoboscidae. This conclusion is based upon finding the oöcysts and sporozoites in flies taken on infected quail, although actual attempts to transmit the parasite by the bite of flies failed, owing to the small number of flies available. One case of transmission was accomplished by injecting sporozoites from an infected fly into the blood of a quail.

3. Transmission was also effected by transplanting a lung of an infected quail into the body cavity of a susceptible quail.

4. All the stages in the life-cycle of the parasite were observed.

   a. Sporozoites were found in oöcysts on the mid-gut, among the tissues of the haemocoele, and in the salivary glands of infected flies.

   b. Schizonts were found in the lungs, liver, and spleen of parasitized birds.

   c. Merozoites were found in endothelial cells of capillaries of the lungs, in epithelial cells of the liver, and, on rare occasions in acute cases, in the peripheral blood.

   d. Gametocytes were found in the red blood cells. Gametogenesis occurred on the microscope slide at room temperature.

5. In microgametogenesis, the gametes are fully formed inside the gametocyte and escape through rupture of the cell membrane.

6. The microgamete is definitely polarized with its nucleus toward the anterior end.

7. The development of the macrogametes is associated with the formation of two polar cells.

8. Fertilization is accomplished in fifteen seconds, the entire microgamete entering the macrogamete. A fertilization membrane appears to be formed immediately after the penetration of the microgamete.

9. The zygote elongates into an oökinete on the slide. On one occasion an oökinete was observed to penetrate the wall of the gut of a fly.

10. The effects of *Haemoproteus lophortyx* on the host vary from infections which appear to be benign to those which are fatal.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Salivary glands of *Lynchia hirsuta*. Iron-haematoxylin stain, photomicrograph, × 80.

Fig. 2. Sporozoites in salivary glands of *Lynchia hirsuta*. Iron-haematoxylin stain, photomicrograph, × 80.

Fig. 3. Oöcysts on wall of mid-gut of *Lynchia hirsuta*. Sketch from living material. × about 350.

Fig. 4. Section of spleen of infected quail showing pigment deposits. Unstained, cleared material, photomicrograph, × 200.

Fig. 5. Section of testis of infected quail showing pigment deposits. Iron-haematoxylin stain, photomicrograph, × 200.

Fig. 6. Smear preparation of lung of infected quail, showing pigment deposits. Unstained, cleared material, photomicrograph, × 80.

Fig. 7. Sausage-shaped schizont from spleen of infected quail. Iron-haematoxylin stain. × 1266.

Fig. 8. Male gametocyte, just preceding liberation of male gametes. Dark field illumination. × about 2500.

Fig. 9. Living microgamete. Dark field illumination. × about 5000.

Fig. 10. Merozoites in epithelial cell from liver. Giemsa's stain. × 1866.

Fig. 11. Sporozoites of *Haemoproteus lophortyx*. Iron-haematoxylin stain. × 3500.

Fig. 12. Sporozoites of *H. columbae*. Redrawn after Adie (1921). × 3500.

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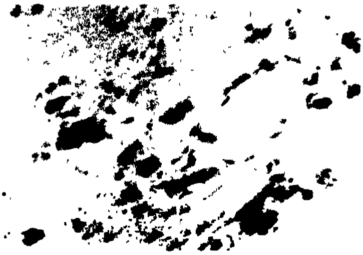
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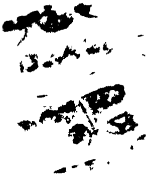
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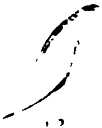
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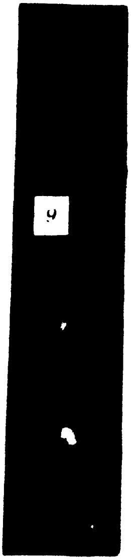
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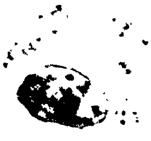
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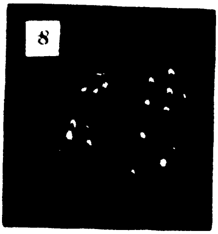
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PLATE 2

All figures drawn with camera lucida, × 2533. Methyl alcohol fixation, Giemsa's stain.

Figs. 13–16. Developing gametocytes.

Fig. 17. Mature male gametocyte in blood cell.

Fig. 18. Mature male gametocyte out of blood cell and rounded up preparatory to gametogenesis.

Figs. 19–23. Successive divisions of the nucleus of the gametocyte resulting in the formation of the nuclei of the microgametes.

Fig. 24. Male gametes in various stages of development. One mature male gamete visible.

Fig. 25. Residual mass of male gametocyte after the liberation of the microgametes.

Fig. 26. Free microgametes.

Fig. 27. Female gametocyte in blood cell.

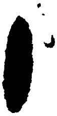
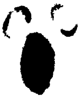
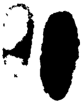
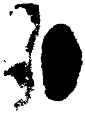
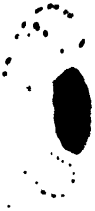
Fig. 28. Female gametocyte outside of blood cell and rounded up preparatory to gametogenesis.

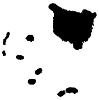
Fig. 29. Macrogamete and polar cell.

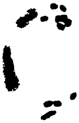
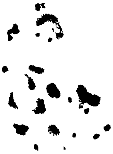
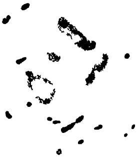
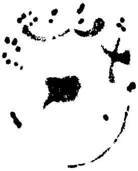
Fig. 30. Zygote with fertilization membrane. Thickness of membrane exaggerated.

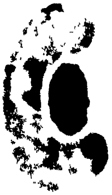
Fig. 31. Elongating oökinete.

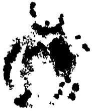
Fig. 32. Motile oökinete.







THE MORPHOLOGY AND LIFE-CYCLE OF OXYMONAS DIMORPHA SP. NOV., FROM NEOTERMES SIMPLICICORNIS (BANKS)

BY

FRANK H. CONNELL

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INTRODUCTION

Of the flagellates found in the hind gut of *Neotermes simplicicornis* (Banks), two have been described: *Hoplonympha natator* Light, 1926, and *Kofoidia loriculata* Light, 1927. The morphology and life-cycle of a third, *Oxymonas dimorpha* sp. nov., are described here. *Oxymonas dimorpha* possesses an anterior prolongation of the cytosome, the rostellum (Kirby, 1928) characteristic of all oxymonads, which in this case is inconspicuous during the motile phase but later functions as an elastic organelle of attachment, often reaching an amazing length. Details of neuromotor structure and the presence of a predominating attached aflagellate phase differentiate *Oxymonas dimorpha* very distinctly from all hitherto described oxymonads.

The following observations were made at the Zoological Laboratory of the University of California. I am indebted to Dr. Charles A. Kofoid and Dr. Harold Kirby, Jr., for advice and helpful criticism.

MATERIAL

Three colonies of *Neotermes simplicicornis* (Banks), collected by Dr. S. F. Light in connection with the work of the Termite Investigations Committee, were supplied at different times during the past year. The life-cycle could not have been worked out had the abundance of living material thus supplied been lacking. The termites were sent to me in the wood in which they were found and were kept in the laboratory in a moist chamber, such as was used by Light and Andrew (1928). One colony was found in a red cedar signal pole, another in a dead mesquite root, and the third in a living *Cholla* root. The first colony was collected near Indio and the second near Niland, Riverside County, California, desert country just north of the Salton Sea, while the third was found in Sabino Canyon, Arizona.

TECHNIQUE

Living preparations were made by teasing out the gut upon a dry slide and applying a cover slip directly to the drop. A drop of 50–50 mixture of 67 per cent Locke's solution and the white of fresh egg was hurriedly applied to the edge of the cover slip, capillary attraction quickly carrying the fluid to and around the intestinal contents. The cover slip need not be sealed with vaseline, as evaporation is slow and the preparation can be studied for an hour without appreciable distortion or mortality.

Intestinal smears were used almost exclusively in preparation of stained material, and since the amount of intestinal fluid is so scanty in *Neotermes simplicicornis,* the whole content of the gut was always used without dilution.

The common fixatives, Schaudinn's, Bouin's, Flemming's, Champy's, and osmic vapor were used, the first being used hot (65° C) as well as cold.

Delafield's haematoxylin, Heidenhain's iron-haematoxylin, safranin, Feulgen's stain, and Mallory's triple connective stain were used with varying degrees of success. Occasionally eosin, light green, and mercurochrome were used as counterstains. Mercurochrome gave a yellowish red color which is trying to the eyes. Delafield's haematoxylin undoubtedly gave the best cytoplasmic differentiation and it was excellent for the structure of resting nuclei. On the other hand, Heidenhain's iron-haematoxylin, following hot Schaudinn's, was by far the best stain for mitotic figures and showed to advantage the structure of the neuromotor apparatus after either Schaudinn's or Champy's. Feulgen's stain colors beautifully the nuclei of all flagellates in the intestine of *N. simplicicornis* save *Oxymonas.* I have, however, found it to have as great an affinity for ingested wood particles as did Kidder (1929) while working upon *Streblomastix strix.* Mallory's triple connective stain promises incomparable results with the larger hypermastigotes and polymastigotes but was of little use in the study of *Oxymonas.*

Dark field illumination was invaluable in determining the size and number of the flagella and in working out the relations of developing neuromotor structures during mitosis.

Artificial production of mitotic flares was successful following defaunation by oxygen pressure as used by Light and Sanford (1928), but the ease with which natural flares were obtained, by daily culling the colonies for newly molted individuals, made such methods unnecessary.

LIFE-CYCLE

The "mitotic flare," following either the molt or infection of artificially defaunated animals, gives rise first to a motile and then, by growth, to an attached, non-motile population. Thus the fluid gut contents of termites which have not recently molted, normally contain neither motile nor dividing forms.

According to Andrews (1928) a period of fasting precedes the molt or ecdysis in *Termopsis angusticollis* during which the xylophagous Protozoa die out, but many of the non-xylophagous polymastigotes persist. When cane sugar is fed to *Neotermes simplicicornis*, all the xylophagous Protozoa except Oxymonas die out. This lives on for extended periods either because wood-eating is facultative or because its large stores of reserve material sustain it. As a practically pure culture of rapidly dividing oxymonads is almost invariably found in the intestine within 48 hours after ecdysis, it is possible that this protozoan remains in the gut throughout the molt. While the original infection during the early instars of the host probably occurs through proctodeal feeding, the very small and infrequent numbers of detached forms found in the normal gut make this method of refaunation of older termites improbable, or of secondary importance. Eating of the cast intima of the hind gut and its contents could not reinfect because it contains neither active nor encysted Protozoa. Isolation of termites which seem about to molt has not solved this question because they either die or fail to molt.

During the mitotic flare which follows ecdysis, nuclear division is rapid in the detached oxymonads. Occasionally the daughter nuclei redivide before plasmotomy is complete; such a condition may be instrumental in the origin of infrequent binucleate individuals. During such rapid division motor organelles, often incompletely formed and non-functional, are discarded at the approach of another nuclear division.

The volutin, with which the attached forms are gorged, becomes exhausted and at its disappearance division ceases, paralleling the case of *Haematococcus pluvialis* noted by Reichenow (1909).

The appearance of motile forms accompanies the reduction in volutin (fig. A; pl. 3, fig. 12). They swarm through the gut and are apparently non-xylophagous for a time. With the reappearance of the hypermastigotes, especially *Kofoidia,* the oxymonads begin to show an elongation of the rostellum (pl. 3, fig. 17) and they become attached in the anterior portion of the hind gut to the intima. Here they form a compact mass nearly filling the lumen of the gut. Wood is again ingested, volutin reappears in an ever increasing amount, degeneration of motor organelles occurs, and the adult attached condition is attained once more.

It will be more convenient to consider the extremes of the life-cycle separately, regarding them as the flagellated or motile phase, and the aflagellated, non-motile, attached or adult phase. It must always be remembered that a perfectly intergraded morphological series may be found running from one extreme to the other toward the end of the mitotic flare.

GENERAL MORPHOLOGY

Motile Phase of the Life-Cycle

The body of *Oxymonas dimorpha*, in the early flagellated phase, is subovoidal or subellipsoidal in shape (fig. A). The rostellum is not yet well developed and forms a low cap of chromophobic fibrillae over the anterior region of the body (fig. A, *ros.*).

The smallest forms are about 17 by 14 microns, though the average of fifty individuals measured before showing any evidence of attachment was 35 by 23 microns, inclusive of the low rostellum. Those individuals which have attained a size of approximately 45 by 25 microns, exclusive of the rostellum, usually show evidence of having become recently attached, the rostellum being often as long as the body.

A thin pellicle, definite, strong, and yet perfectly elastic, covers the body. Many spiral bacteria, attached by one end, adhere to the pellicle. They are most numerous on the posterior third of the body, probably because this is partly protected as the organism swims through the intestinal contents. The type of bacteria adhering to the body varies with the stage of the life-cycle. As volutin begins to appear in the cytoplasm, the spirals are largely displaced by a rod which literally covers the surface of the body (pl. 3, fig. 18).

There is no cytostome. Solid food is not ingested in the motile phase. After attachment, however, wood particles are ingested through the pellicle.

The posterior cytoplasm is finely alveolar and contains numerous fine, weakly siderophile granules. Supporting the axostyle centrally and running antero-laterally to the base of the rostellum is a mass of hyaline, non-alveolar material later referred to as the *clear area* (fig. A, *cl. ar.*). It is demarked sharply from the posterior alveolar cytoplasm. It is unlike the *sleeve* described in other species of *Oxymonas* in both structure and function. In living material that has begun to disintegrate somewhat, the posterior cytoplasm occasionally pulls away posteriorly from the clear area, leaving it in place. It can then be seen that the clear area is of a firmer consistency than the posterior cytoplasm. Thus its function seems to be one of support for the neuromotor system during the short period of motility of the organism. After attachment, when the power of motility has been lost, the clear area disappears along with the degeneration of flagella and blepharoplasts (fig. B).

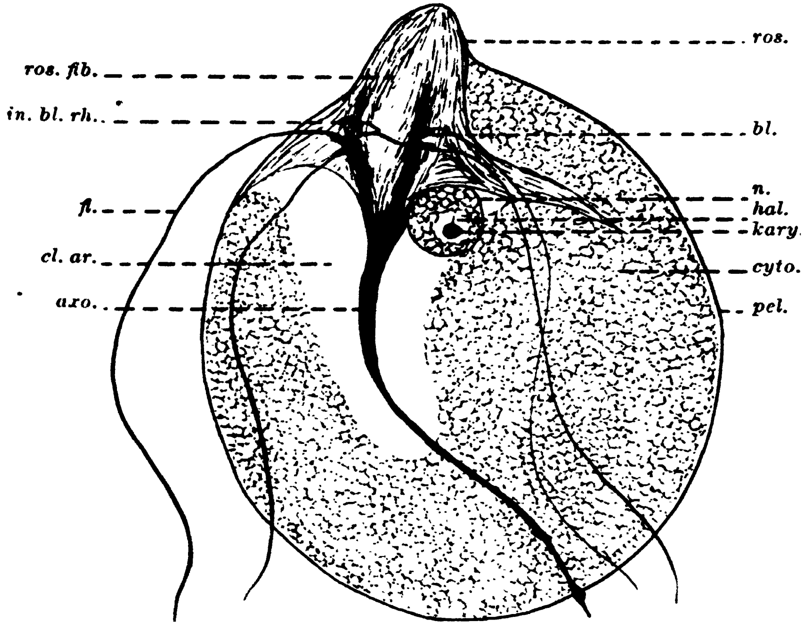

Fig. A. Motile phase of *Oxymonas dimorpha* sp. nov. Drawn with camera lucida, × 3500. *Axo.*, axostyle; *bl.*, blepharoplast; *cl. ar.*, clear area; *cyto.*, cytoplasm; *fl.*, flagella; *hal.*, halo; *in. bl. rh.*, inter-blepharoplast rhizoplast; *kary.*, karyosome; *n.*, nucleus; *pel.*, pellicle; *ros.*, rostellum; *ros. fib.*, rostellar fibrillae.

During the motile phase the nucleus is found usually within the clear area and in direct contact with the fibrillae of the rostellum. The diameter of the nucleus ranges from 4 microns, found in a small motile form, to 12.3 microns in a large attached individual. The average diameter of the nucleus, taken from measurements of seventy-five individuals of both types, was 7.7 microns.

The nuclear membrane is delicate and within it is found the posteriorly excentric karyosome (fig. A, *kary.*) or "Binnenkörper" of Janicki (1915). The karyosome itself is surrounded by a clear halo (fig. A, *hal.*). A peripheral reticulum of fine chromatin granules completes the component structures of the nucleus. In most nuclei the karyosome sends out faint spoke radii to the reticulum. The karyosome averages about one-third the diameter of the nucleus in size and is strongly basophilic, though the central portion does not always stain evenly. No lateral granule or entosome in the halo as described by Janicki (1915), nor centrosomes on the nuclear membrane as described by Kofoid and Swezy (1926), for *O. granulosa*, have been noted.

The neuromotor apparatus of *Oxymonas dimorpha* consists of two sets of two stout antero-lateral flagella, usually longer than the body (fig. A, *fl.*), arising from two antero-lateral blepharoplasts (fig. A, *bl.*) which are located near the anterior tip of the axostyle. The blepharoplasts are occasionally interconnected by a heavy basophilic rhizoplast (fig. A, *in. bl. rhiz.*) as in the type species. Its structure seems to be that of fused rostellar fibrillae like the axostyle, and it may act as a brace between the blepharoplasts. Owing to the short motile period in the life-cycle of *O. dimorpha*, it is transient in nature and in most individuals functions for a very short time.

The axostyle (fig. A, *axo.*) is strongly basophilic. Anteriorly it is continuous with the fibrillae of the rostellum from which it arises during the anaphase of mitosis. It is curved, irregularly axial, and often protrudes posteriorly three or four microns beyond the body, ending in a fine, arrowhead-shaped structure.

The rostellum (fig. A, *ros.*) is composed of many chromophobic fibrillae which form a cap over the anterior region of the body and which are attached to the pellicle antero-laterally in fine strands, which later become the supporting fibrils of the attached phase (fig. B, *s.fib.*). The fibrillae first appear during the anaphase, surrounding the nuclear membrane. They not only seem to arise from it, but also to retain their attachment to it until well into the attached phase. The attachment is shown to advantage only in individuals which have been distorted by smearing. The rostellum may be swung from side to side, rather feebly, during the late motile phase (pl. 3, fig. 17) but after attachment, when the clear area has disappeared, it is no longer actively motile and is not even drawn back into the body after stimulus with a fine needle (pl. 3, fig. 19).

Parabasal bodies have not been found after fixation in either Flemming's or Champy's fluid.

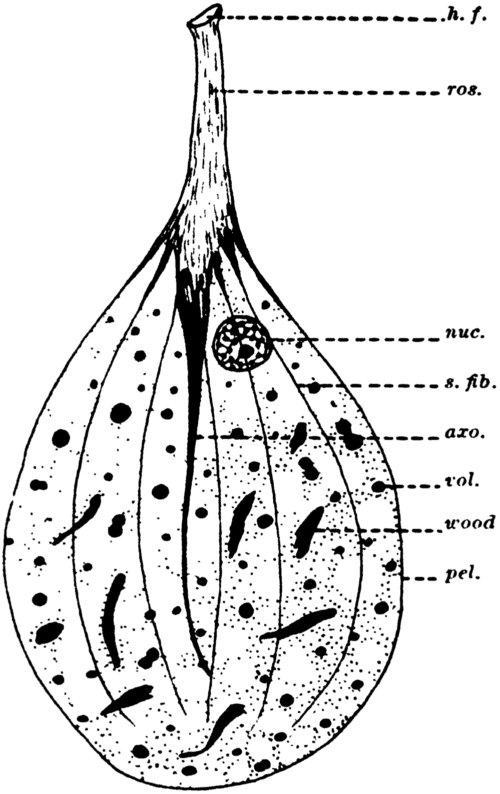

Fig. B. Attached phase of *Oxymonas dimorpha* sp. nov. Drawn with camera lucida, × 850. *Axo.*, axostyle; *h.f.*, hold-fast; *nuc.*, nucleus; *pel.*, pellicle; *ros.*, rostellum; *s. fib.*, supporting fibrils; *vol.*, volutin; *wood*, wood.

ATTACHED PHASE OF THE LIFE-CYCLE

Attachment of motile oxymonads to the intima of the gut is practically complete by the time the hypermastigote fauna has returned to normal. Termites lacking a hypermastigote fauna (for example those fed on a cane sugar diet) contain both motile and non-motile forms regardless of the time which has passed since ecdysis. Is there some intergeneric relationship which impels the oxymonads to attach themselves in the anterior portion of the hind gut which, with respect to the food supply, is certainly the most favorable of ecologic niches?

Attachment, limiting as it does free movement, brings about marked morphological and physiological changes which better adapt the organism to this new mode of existence. Most obvious of these changes is the elongation of the rostellum, which now functions as an elastic organelle of attachment (fig. B, *ros.*). At the tip, a holdfast, flattened or slightly cupped, is applied directly to the intima of the gut (fig. B, *h.f.*). The rostellum is expanded at the base; strands of fibrillae (fig. B, *s. fib.*) fuse in a manner comparable to the fusion of the rostellar fibrillae in the formation of the axostyle, and run as supporting fibrils beneath the pellicle or occasionally into the depths of the body as if supplementing the axostyle. In the formation of supporting fibrils the rostellar fibrillae undergo a reversal of staining properties and like the axostyle, become basophilic. The flagella, blepharoplasts, and clear area disappear, leaving the axostyle connected with the rostellum by two or three basophilic fibers. The axostyle no longer protrudes from the body but ends freely in the cytoplasm; its fine arrowhead-shaped point usually disappears. The body tends to be piriform but is distorted by contact with neighboring organisms and by smearing. The rostellum varies in length and is extremely elastic, one having been traced 1250 microns without a break. The size of the body, exclusive of the rostellum, averages about 120 by 100 microns, but one individual measured 190 by 165 microns.

The cytoplasm becomes thickly scattered with ingested wood particles of various shapes and sizes. Vacuoles, large and small, and containing indeterminate substances, are often present. Spherules of volutin or metachromatin appear in ever increasing numbers and are dispersed throughout the cytoplasm. Janicki (1915), noting comparable spherules in *O. granulosa,* suggests that this material consists of wood particles which have been molded into shape by the cytoplasm. Grassi and Foa (1911), working with *Microrhopalodina,* regard them as reserve products. Feulgen's stain supports the latter view, for it stains the wood particles and leaves the volutin colorless.

MITOSIS

The post-flare protozoan population of the termite intestine seems to be very stable once the normal intergeneric ratio has been established. Mitotic figures are found in the normal termite only during the first few days after ecdysis.

Division seems to occur only in individuals which contain volutin and continues until it has disappeared. Apparently division is very rapid. An individual, noted under dark field illumination, divided within seven minutes after two functional neuromotor systems appeared in the cell. Several individuals have been found containing four nuclei, all in the anaphase. Many others have been found with two redividing nuclei, which leads one to suspect that nuclear division tends to be more rapid than division of the cytoplasm. If this be the case, it is small wonder that the termite gut is teeming with oxymonads before other protozoa become greatly increased in number.

Long before ecdysis, the nucleus abandons its connection with the rostellar fibrillae and lies freely in the cytoplasm (pl. 3, fig. 14). Little or no wood is ingested during the flare, probably because the termite itself has eaten very little. The axostyle, rostellum, and supporting fibrillae are resorbed.

The prophase is characterized by the migration of the karyosome to the center of the nucleus where it elongates (pl. 3, figs. 2 and 3). The peripheral chromatin becomes clumped into larger aggregates and, as the tips of the elongating karyosome reach the nuclear membrane, most of the peripheral chromatin comes to lie in beads midway between the poles of the karyosome which has now formed the centrodesmose (pl. 3, figs. 3 and 4). Some peripheral chromatin does not enter into the formation of chromosomes but persists throughout mitosis, lying beneath the nuclear membrane. In some cases fine fibrils run radially from the peripheral granules toward the spindle (pl. 3, fig. 5).

From the wealth of mitotic figures in my material, Janicki's suspicion that the centrodesmose arises from the karyosome can be easily confirmed. In small nuclei, and occasionally in large ones (pl. 3, fig. 6), it remains axial throughout mitosis, but in most nuclei it soon bends and comes to lie just inside the nuclear membrane (pl. 3, fig. 5). Owing to its original axial position, the writer follows Kirby in applying the word centrodesmose here, though this word is not accurately descriptive for mitotic stages later than the anaphase.

A well developed spindle forms between the poles of the centrodesmose, upon the equatorial plate of which the beaded chromatin comes to lie (pl. 3, fig. 4). It is not until the anaphase, however, that the individual chromosomes can be seen.

The extreme abundance of anaphase figures may indicate that this stage is rather more prolonged than other stages in mitosis. The nucleus begins to elongate, pushing out slightly at the poles of the centrodesmose from which the chromosomes radiate (pl. 3, figs. 5

and 6). Owing to the bending of the centrodesmose, the spindle has, by this time, come to lie along its concave side.

The new neuromotor system first becomes evident at about this time. The nucleus lies in an area of clear, homogeneous material at the outer edge of which, and approximately opposite each pole of the centrodesmose, the new axostyles appear as small basophilic rods (pl. 3, fig. 9). Apparently it is this clear, undifferentiated substance which, under dark field illumination, is more refractive to light than the rest of the cytoplasm that gives rise to the whole neuromotor system. The writer has found no evidence for the origin of such organelles from extra-nuclear bodies nor from bodies extruded from the centrodesmose (endosome) as in *Euglena* (Baker, 1926). The axostyle does not, as Janicki suspected, arise from that part of the centrodesmose left outside the nuclear membrane at the telophase (pl. 3, fig. 11).

The centrodesmose, bent to a semicircle in the anaphase, continues to elongate. As the nucleus lengthens along the axis of the spindle, its membrane constricts and the centrodesmose, slightly spoon-shaped at its tips, straightens out to the position shown in plate 3, figure 7. The chromosomes remain intact while the neuromotor system grows (pl. 3, fig. 10) and becomes functional (pl. 3, fig. 11). Although plasmotomy occurs very soon, the nucleus is reorganized before it is complete. The peripheral reticulum seems to arise from the chromosomes and the karyosome from the spoon-shaped tip of the centrodesmose, which remains within the nucleus. The remainder of the centrodesmose, excluded from the nucleus, remains attached at each end to the rostellar fibrillae, breaks unequally near the middle, persists only a short time as a sort of small, secondary axostyle, and is gradually resorbed.

The blepharoplasts and flagella form near a fork at the anterior tip of the axostyle. Occasionally the extranuclear portion of the centrodesmose appears to remain attached to one of the blepharoplasts for a short time. When division is rapid, as is evident in multinucleate individuals, the growing neuromotor systems are discarded by the nucleus before they become functional and the nucleus again divides.

INTRACYTOPLASMIC PARASITES

Two distinct types of intracytoplasmic parasites have been found occasionally in *O. dimorpha* and in no other inhabitant of the gut. Neither has been noted free in the gut. Both are of uncertain systematic position.

One is slender, pointed at each end, and contains several large chromatic granules evenly distributed throughout its length (pl. 3, fig. 15). These lie parallel to each other in small bundles (pl. 3, fig. 20) which suggests the result of longitudinal division. Their size is about 15 by 2.5 microns.

The other (pl. 3, fig. 16) is stouter than the first and is pointed at each end. It contains a definite pycnotic nucleus like so many intracellular parasites, which seemingly divides amitotically (pl. 3, fig. 16). It assumes no regular arrangement within the cell and its size is about 17 microns by 3 microns.

Apparently neither parasite is particularly pathogenic to the host, though such parasitized individuals as have been found have nuclei which are poor in peripheral chromatin.

DIAGNOSIS

Figures A and B

Oxymonas dimorpha sp. nov.

Motile phase.—Shape subovoidal. Pellicle delicate. Cytoplasm without wood or volutin. Siderophile axostyle, protruding posteriorly, anteriorly supported by the clear area and fusing with the rostellar fibrillae. A pair of long, stout, antero-lateral flagella from each of two antero-lateral blepharoplasts. Blepharoplasts usually interconnected by a rhizoplast. Rostellum fibrillar, low, and mobile. Nucleus, connected anteriorly with rostellum, contains a posteriorly excentric karyosome with spoke radii running through a clear halo to a fine peripheral reticulum.

Attached phase.—Gradual elongation of rostellum and attachment by holdfast to intima of hind gut. Rostellar fibrillae fuse to form siderophile, subpellicular supporting fibrils. All motor organelles, save axostyle and rostellum, disappear. Cytosome becomes gorged with volutin and wood. Shape piriform.

Size range from motile to attached forms is 17 by 14 microns to 195 by 165 microns.

In the possession of four long flagella *Oxymonas dimorpha* resembles those species of *Oxymonas* noted by Kirby (1928) in Central American *Kalotermitinae*, *K. tabogae*, *K. marginipennis*, and in the California representatives of the family, *K. hubbardi* and *K. minor*. The flagella number of the type species, *O. granulosa*, and of three species of *Oxymonas* from South American termites, have been reported as six by Kofoid and Swezy (1926).

Oxymonas dimorpha differs from all other described members of the genus in its great size, ranging up to 195 × 165 microns, and in details of neuromotor structure. It differs from all other *Oxymonadidae*, with the possible exception of *Microrhopalodina*, in the complete degeneration of motor organelles during the attached phase.

Host: Neotermes simplicicornis (Banks) from Arizona and Southern California.

DISCUSSION

Evolution.—Phylogenetic relationships among the polymastigotes have been based largely upon basic types of neuromotor systems, from which have occurred variations, such as increase or modification of flagella and multiple duplication of thus modified types. In the *Devescovinidae*, there has been a progressive increase in the size of the trailing flagellum accompanied by a fusion of the pellicle to form eventually an undulating membrane. In the *Calonymphidae*, on the other hand, there has been a multiplication of neuromotor systems without modification of the basic type. It has been suggested that *Oxymonas* has arisen by a partial duplication of a "fundamentally triflagellate type" of neuromotor system, such as that of *Monocercomonas*. If, however, reinvestigation shows previously described species to agree in flagella number with those examined by Kirby (1929) and the writer, such an assumption cannot be true.

It would seem that more reliance should be placed upon fundamental differences in mitotic phenomena than upon variation in motor organelles which, owing to their direct contact with the environment, should and do show wide variation in number, size, and arrangement. Thus in the trichomonads the flagella number is a variable, but the paradesmose, arising from the centroblepharoplast during mitosis, is a constant. In the *Euglenida*, also, flagella number is a variable, yet the obvious relationships are substantiated by similar mitotic phenomena. If such logic be sound, no close relatives of the oxymonads are known among either parasitic or free-living flagellates.

Within the family the path of evolution is more easily traced. All adaptations seem to have been dominated by a tendency ever to increase the speed of reproduction. Attachment by means of the rostellum gained advantage of position within the gut and made possible great body size. With increase of body size the capacity for storage of reserve products, which make rapid division possible, is also augmented. Such specialization has resulted, however, in degeneration of motor organelles, during the attached phase. It is interesting to note that at least one multinucleate oxymonad, *Microrhopalodina,* also loses its motor organelles after attachment (Grassi, 1911). The repeated recurrence of flagellated forms during the flare and growth to the non-motile attached forms following the flare lead one to interpret the life-cycle of *Oxymonas dimorpha* as an illustration of the Biogenetic Law.

Nucleus.—The nucleus, described above, exhibits peculiarities of reaction to Feulgen's which is quite without parallel among animal cells and merits consideration here. Though the nuclei of all protozoans in the gut of *Neotermes simplicicornis* stain perfectly by this method, no variation of technique has been successful with *Oxymonas.* The tiny nucleus of *Hoplonympha* stains more perfectly than by any other method, yet the nucleus of Oxymonas persistently refuses to react. Counterstaining fails to show any sign of effect by Feulgen's.

Karyosome.—The karyosome, though containing both chromatin and plastin in the resting phase, is not divided equally between the daughter nuclei as in the *Euglenida,* but forms the centrodesmose which appears to be comparable functionally to the paradesmose of the polymastigotes. The greater part of it remains outside the nucleus following the telophase, the spoon-shaped tips alone remaining to form anew the karyosome of the daughter nuclei.

Axostyle.—The method of formation of the axostyle in *Oxymonas* seems to be peculiar to the family and throws little light upon the origin of that organelle in other protozoans. It appears first as a tiny basophilic rod near but not connected to the nucleus save by rostellar fibrillae. It seems to arise by fusion of fibrillae, and its further growth probably occurs by progressive differentiation of the original small fused portion. Origin from the centrodesmose is definitely excluded as the axostyle is half-formed while the centrodesmose is still contained entirely within the nuclear membrane. Complete resorption of the parental motor organelles long before mitosis and the fact that the nucleus lies free in the cytoplasm at division exclude any possibility of origin from the neuromotor system of the parent.

Volutin.—The writer has applied the term *volutin* to a metabolic product of the cell which, in the attached phase, is found in scattered spherules throughout the body, exclusive of the rostellum. It appears only after ingestion of wood. The total volume increases steadily until the flare, during which is it rapidly reduced. Mitotic figures have not been found in individuals which lack it. It does not stain with iodine and osmic acid does not blacken it. Feulgen's does not react with it but its affinity for basic dyes is as great as that of true chromatin. Thus in every way this substance is comparable to material found in other Protozoa, and usually termed volutin or metachromatin. Its behavior during mitotic flares exactly parallels that of a similar substance found by Reichenow in *Haematococcus pluvialis.*

SUMMARY

1. *Oxymonas dimorpha* sp. nov. inhabits the hind gut of *Neotermes simplicicornis* (Banks).

2. Individuals which have become attached to the intima of the gut probably become detached and remain in the gut at ecdysis, giving rise, by repeated division, to smaller flagellated forms. The flagellated forms are at first non-xylophagous, but gradually adopt this diet. The rostellum elongates and the organism becomes attached to the gut by a hollow, cup-shaped holdfast which develops at its tip. The attached forms become gorged with wood particles and volutin; the motor organelles degenerate.

3. Volutin disappears slowly during the mitotic flare and division ceases when it is depleted.

4. All stages of mitosis are described.

5. A centrodesmose arises from the karyosome.

6. The axostyle does not arise as an extranuclear remnant of the centrodesmose, but from fused rostellar fibrillae which seem to arise from the nuclear membrane.

7. All motor organelles are formed anew at each division of the nucleus.

8. Two intracellular parasites of uncertain systematic position are described and figured.

9. *Neotermes simplicicornis* fed upon a cane sugar diet retains a fauna of polymastigote protozoans including *Trichomonas, Janickiella,* and *Oxymonas.* In the case of the latter, wood-eating may be facultative or due to use of large amounts of reserve material. *Spironympha* is the only hypermastigote which remains.

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EXPLANATION OF PLATE

All figures, except 9, 10, 11, 15, and 16 were made by camera lucida from smears fixed in hot Schaudinn's fluid and stained in iron haematoxylin.

PLATE 3

Fig. 1. Resting nucleus with spheroidal karyosome, spoke radii, halo, and peripheral chromatin reticulum. × 2350.

Fig. 2. Surface view of early prophase with karyosome elongating. × 2350.

Fig. 3. View of same nucleus in optical section. × 2350.

Fig. 4. Metaphase with beaded chromatin on the equatorial plate. Karyosome now forming the centrodesmose. × 2350.

Fig. 5. Typical anaphase with the centrodesmose bent to one side. ×2350.

Fig. 6. Anaphase in which the centrodesmose has remained axial. × 2350.

Fig. 7. Telophase with daughter nuclei connected by straightened centrodesmose. × 2350.

Fig. 8. Reorganization of the karyosome in daughter nucleus. The developing neuromotor system, omitted in the earlier figures, is included here to show the fate of the centrodesmose, which is seen as the curled fiber running posteriorly from the left blepharoplast. × 2350.

Fig. 9. First appearance of the new neuromotor systems in the anaphase. The small basophilic rods are the new axostyles. Semidiagrammatic.

Fig. 10. Later stage showing growth of the axostyle. Semidiagrammatic.

Fig. 11. Functional neuromotor systems at a stage corresponding to figure 8. Semidiagrammatic.

Fig. 12. Motile form arising from rapidly dividing detached forms. × 1350.

Fig. 13. Parasitized motile form, × 1350. See fig. 16.

Fig. 14. Attached form with degenerated motor organelles. The nucleus, having lost its attachment, has migrated posteriorly and, lying free in the cytoplasm, awaits mitosis. × 685.

Fig. 15. Parasites drawn from individual in figure 20. Semidiagrammatic.

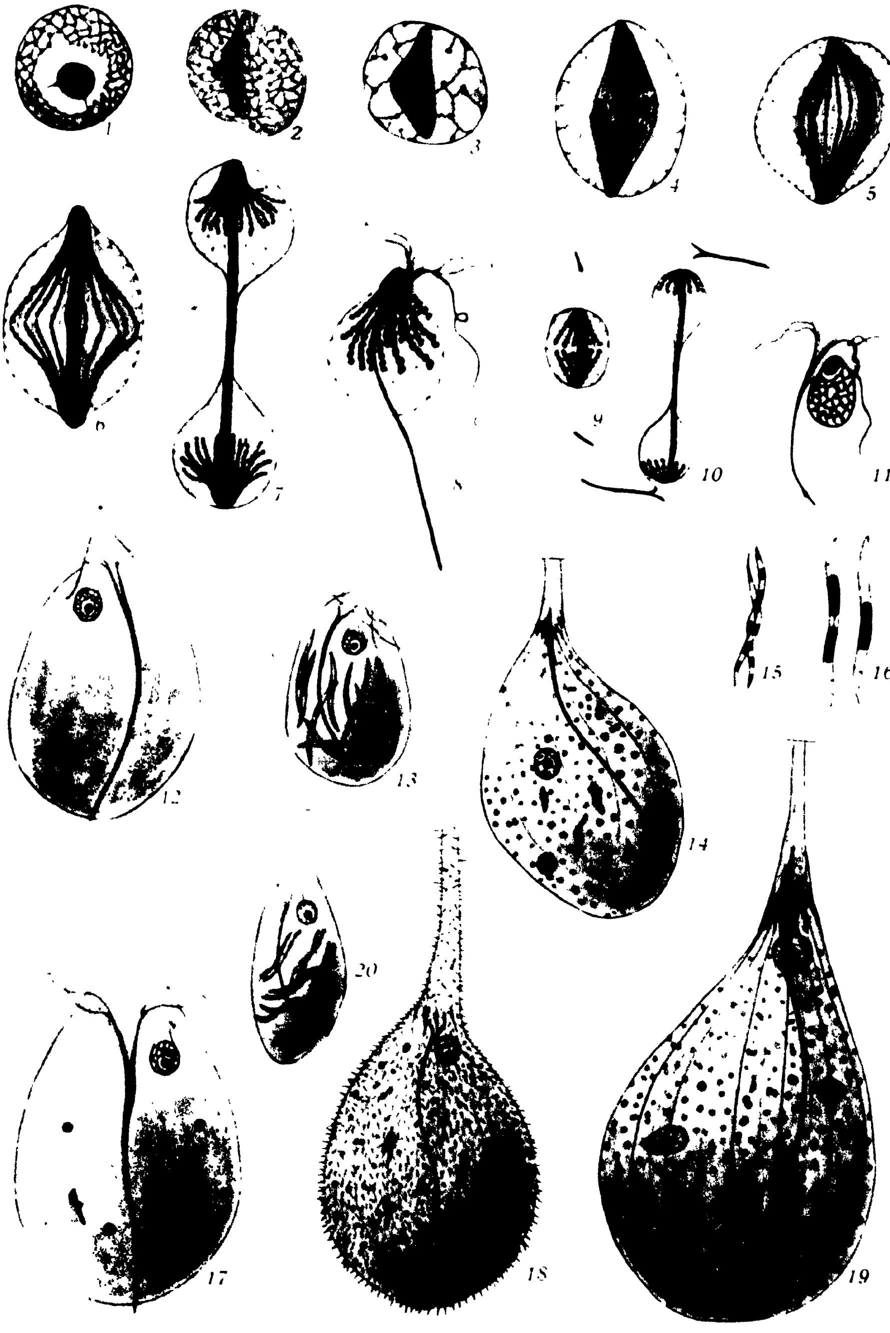
Fig. 16. Parasites drawn from individual in figure 13. Semidiagrammatic.

Fig. 17. Motile form with elongate rostellum but without showing signs of attachment. × 1350.

Fig. 18. Attached form showing the bacteria which cover the pellicle. × 685.

Fig. 19. Attached form showing degeneration of motor organelles and supporting fibrils. Ingested wood particles and spherules of volutin fill the body. × 685.

Fig. 20. Small parasitized attached form, × 685. See figure 15.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HYPERMASTIGOTE FLAGELLATES FROM THE TERMITE RETICULITERMES: TORQUENYMPHA OCTOPLUS GEN. NOV., SP. NOV., AND TWO NEW SPECIES OF MICROJOENIA

BY

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HYPERMASTIGOTE FLAGELLATES FROM THE TERMITE RETICULITERMES: TORQUENYMPHA OCTOPLUS GEN. NOV., SP. NOV., AND TWO NEW SPECIES OF MICROJOENIA

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INTRODUCTION

Several small flagellates have been described by various workers in the past as the young of the larger flagellates of termites. Leidy (1881) observed and figured what he believed to be immature stages of *Trichonympha agilis* from *Reticulitermes flavipes.* The smallest of these measured from 9 to 12 microns in length; these were delicate and lively in their motion, and possessed a thick investment of flagella. It is possible that Leidy's figures 15 and 16 of plate 1 represent flagellates belonging to the genus *Torquenympha* described in this paper. Duboscq and Grassé (1928) have described stages of *Spirotrichonympha flagellata* Grassi from *Reticulitermes lucifugus* and *Spirotrichonympha kofoidi* Duboscq and Grassé from *Reticulitermes flavipes.* They believe that the genera *Microjoenia* Grassi and *Spironympha* Koidzumi (= *Microspironympha* Koidzumi) are stages in the life-cycle of *Spirotrichonympha*, and have endeavored to show that intermediate stages exist between each of these forms and the typical *Spirotrichonympha.*

In addition to *Trichonympha, Holomastigotes, Spirotrichonympha,* and the Pyrsonymphidae, *Reticulitermes hesperus* contains a number of smaller flagellates. It is the belief of the writer that these represent three independent species falling into genera corresponding to *Spironympha, Microjoenia,* and *Torquenympha. Torquenympha* has been figured by Duboscq and Grassé (1928, fig. 6) as a young stage in the life-cycle of *Spirotrichonympha kofoidi.*

The flagellates of *Reticulitermes hesperus* Banks have been reviewed by various workers (Kirby 1924, Powell 1928). Several small flagellates occur in the termite and among them *Microjoenia* has been identified.

Two species have been described in this genus: *Microjoenia hexamitoides* was reported in *Reticulitermes lucifugus* Rossi by Grassi (1892) and *Microjoenia axostylis* was described by Cutler (1920) from *Archotermopsis wroughtoni* Desneux.

Recently Duboscq and Grassé (1928) have reported *Microjoenia* stages to occur in the life-cycle of *Spirotrichonympha.* They also described intermediary stages between *Microjoenia* and *Spirotrichonympha;* therefore they conclude that *Microjoenia* should be considered as a form in the life-cycle of *Spirotrichonympha* and that *Microjoenia* should not be considered as a valid genus. The writer sees very little evidence for this decision and believes that these intermediary stages described by Duboscq and Grassé (1928) should be placed in the related genera, *Torquenympha* and *Microjoenia.*

The work here reported was done in the protozoological laboratory of the University of California. I wish to express to Dr. C. A. Kofoid and to Dr. Harold Kirby, Jr. many thanks for their valuable criticisms and assistance in the preparation of this paper, and to the former for granting access to his extensive library of protozoological literature.

The material was obtained from *Reticulitermes hesperus* Banks collected from an old oak log on the University campus, and from *Reticulitermes hageni* Banks collected near Glenford, Georgia. After the termites were procured they were placed on a filter paper diet, as suggested by Kirby (1926). Studies were made from both living material and fixed preparations. The best fixing solutions were found to be Schaudinn's used hot, Flemming's strong without acetic and Champy's solution. Heidenhain's iron-haematoxylin gave the most satisfactory nuclear stain, and counterstains of eosin and light green were used for cytoplasmic structures. Osmic vapor aided in demonstrating the axostyle, parabasal bodies, and the flagella.

Torquenympha octoplus gen. nov., sp. nov.

Plate 4

In *Reticulitermes hesperus* the small flagellates described under this name are not so abundant as the other hypermastigotes. They are easily recognized in the living material by their quick, jerking method of locomotion. The generic name *Torquenympha* (*Torque,* a small necklace, and *nympha,* a nymph) has been selected for this flagellate because the parabasal bodies lie in a circle like a tiny necklace around the anterior portion of the animal, though buried in the cytoplasm. The flagellate has a single nucleus and axostyle, but the other structures number eight or a multiple of eight; hence the specific name *octoplus* has been given to it.

The body is top-shaped, or pyriform, measuring 20 (15–26) microns in length and 11 (9–13) microns in width. It is never flattened on one side like *Microjoenia.* Also this flagellate does not move its anterior end about as do the related forms, *Spironympha* and *Microjoenia.*

The neuromotor system consists of the centroblepharoplast, sixteen to twenty-four anterior flagella, a rhizoplast, an axostyle, and a parabasal ring. The flagella are attached in a radial manner to the basal granules situated at the margin of the centroblepharoplast. These are about equal to the body in length, measuring 18 to 22 microns. The centroblepharoplast is large and has a deeply staining cap. A nuclear rhizoplast is attached to the base of the centroblepharoplast and extends into the endosome of the nucleus. The axostyle is a fibrous, rod-like structure connected to the centroblepharoplast and enclosing the nucleus. It extends posteriorly through the endoplasm to the posterior end of the body, where it terminates in an acute point. Quite often the axostyle is split posteriorly into several small filaments which end freely in the cytoplasm. It is similar in structure to the axostyle described in *Microspironympha elegans* by MacKinnon (1927). Its dispersed nature suggests that its primary function is not that of an axial supporting structure, but that it also seems to aid in holding the nucleus in position.

Surrounding the nucleus there are eight ovoidal parabasal bodies, which stain deeply after osmic acid fixation. These are suspended from the centroblepharoplast by fine threads, and each is connected laterally to the adjacent parabasal bodies by slight, granular strands. There is thus a circle of parabasals at the level of the nucleus, interconnected to form a ring. Beyond the end of each of the parabasals is a slender strand which extends downward into the cytoplasm in a leiotropic spiral.

The nucleus is ovoidal in shape and contains a large spherical endosome surrounded by a hyaline area. It is anterior in position and is surrounded by the fibers of the axostyle. The operculum, a clear rounded cytoplasmic area at the tip of the body, is only slightly developed in *Torquenympha.*

The endoplasm is vacuolated and slightly granular. The food particles, which are never of large size, are usually collected in the lower two-thirds of the body. *Torquenympha* is xylophagous. In living material under unfavorable conditions, portions of the cytoplasm are cast off from the posterior part of the body. This behavior can be induced by a change in the medium in which the flagellates swim.

THE GENUS MICROJOENIA

The genus *Microjoenia* has been described by Grassi and Foa (1911) as follows:

> Flagelli sorgenti in fitte serie sub-longitudinali da una zona vicina all'extremità an anteriore delimitante preciò quivi un cherchio anteriore, il quale si presenta come l'operculo di un augusta lacuna contenente liquido, e risalta percè sprovisto di flagelli: bastoncello assile: una serie di speciali corpi tondeggianti al margine posteriore della zone suddetta; fore puittost piccole.

Cutler (1920) described *Microjoenia axostylis* as being closely related to *Microjoenia hexamitoides* Grassi. It is 16 to 30 microns in length and 12 to 25 microns in width. At the anterior end there is a tuft of flagella which arise from a series of granules arranged in two or three longitudinal rows. He finds that the anterior end of the body is not raised into a papilla, but is much flattened and thickened. He finds no evidence for the operculum or ''lake'' containing fluid, as described by Grassi. The animal has a large axostyle which is 4–5 microns wide. From the above descriptions it is obvious that the animals I shall describe should be placed in the genus *Microjoenia.*

Microjoenia ratcliffei sp. nov.

Plate 5, figures 10, 12–15, 17

The body length is 18–26 microns; the width is 12–16 microns. The body shape varies from pyriform to top-shape. It is flattened on one side near the anterior end as in *Microjoenia axostylis* Cutler. The posterior end is sharply pointed. The host is *Reticulitermes hesperus* Banks.

The anterior flagella are sixteen to twenty in number and they are attached to the base of the centroblepharoplast and the longitudinal rows of basal granules.

The centroblepharoplast of this species is large and blunt. By no means is it as large as that of *Microjoenia axostylis* Cutler; in structure and size it is nearer that of *Microjoenia hexamitoides* Grassi.

The anterior nucleus is oval and it has a large central mass of chromatin, the endosome. This structure is often granular. The rhizoplast suspends the nucleus from the centroblepharoplast.

The parabasals are usually two in number; these lie on either side of the nucleus and they are suspended from the base of the centroblepharoplast by small strands (figs. 12 and 16, pl. 4). They are not so numerous as the parabasals of the *Microjoenia* stages described as a part of the life-cycle of *Spirotrichonympha* by Duboscq and Grassé (1928). Also the writer sees no reason to believe that the parabasals of this flagellate are homologous to the Golgi apparatus, as Duboscq and Grassé believe; *Microjoenia* has Golgi bodies similar to those described by Hirschler (1914); that is, the Golgi apparatus appears as rings, crescents, and spheres when treated with osmic acid. These seem to act independently of the parabasal bodies in every way (figs. 15 and 16, pl. 5).

The axostyle of this species is not so wide as it is in *Microjoenia axostylis* Cutler; it is narrow and contains a thread-like filament. At the base of the nucleus are a series of granules which are suspended from the fibers of the axostyle (figs. 10, 13, 16, pl. 5).

The number of longitudinal lines of basal granules is greater than in *Microjoenia hexamitoides* Grassi or *Microjoenia axostylis* Cutler; they are fifteen to eighteen in number. These longitudinal strands begin at the base of the centroblepharoplast and extend posteriorad for one-fourth of the body length.

Microjoenia pyriformis sp. nov.

Plate 5, figures 11, 16

This is the smallest hypermastigote in *Reticulitermes hageni* Banks. The body shape is pyriform; the length 44–52 microns; the width is 24–30 microns. It is not so flattened as in the other species of *Microjoenia*. It is the largest described form of this genus. Its posterior end is not so sharply pointed as that of the other described species.

The anterior flagella are eighteen to twenty in number, and are attached to the basal granules or to the centroblepharoplast. These longitudinal rows of granules are more pronounced than in *Microjoenia ratcliffei* or *Microjoenia axostylis* Cutler.

The anterior nucleus has a granular endosome. The chromatin granules are rarely compact, as they are in the other species, and the nucleus is more ellipsoidal than that of *Microjoenia axostylis* Cutler or *Microjoenia hexamitoides* Grassi.

The centroblepharoplast is prominent and extends farther anteriorad than that of the other described species. It may show a clear center similar to that of *Microjoenia hexamitoides* Grassi (figs. 11 and 15, pl. 5).

I have been unable to stain the parabasals of this species. The axostyle is small and it is by no means so wide as that of *Microjoenia axostylis Cutler.* It does not contain the granules which are at the base of the nucleus in *Microjoenia ratcliffei.* The longitudinal lines are twelve to sixteen in number.

DISCUSSION

Duboscq and Grassé (1928) have described young stages of *Spirotrichonympha* which they believe to be comparable to *Microjoenia.* They describe *Microjoenia* stages with 6, 8, 9 parabasals and also stages with 8 to 20 parabasals which form a ring around the apex of the animal. The writer believes that the first group should go into the genus *Microjoenia;* whereas those of the second group, because of their shape and parabasal ring, would probably correspond to *Torquenympha.* The last series of so-called young stages probably belongs to *Spironympha* Koidzumi (=*Microspironympha*). These are the forms which Duboscq and Grassé describe as a stage in the life-cycle of *Spirotrichonympha,* which has a definite apex with spiral lines instead of longitudinal lines, and numerous parabasal bodies which are arranged in a spiral manner. The longitudinal lines, they believe, later become spiral like those of *Spironympha* forms. It is rather difficult to believe that the longitudinal basal granules can be transformed into the spiral flagellar bands of *Spironympha.* These longitudinal rows of basal granules are absent in *Torquenympha* and they are not shown in the corresponding forms figured by Duboscq and Grassé (1928).

The writer does not see enough evidence to persuade him to consider *Microjoenia* and *Spironympha* (=*Microspironympha*) as forms in the life-cycle of *Spirotrichonympha;* and he believes that most of the intermediary stages described by Duboscq and Grassé (1923) fall, possibly, within the genus *Torquenympha.*

DIAGNOSES

Torquenympha gen. nov.

A small xylophagous hypermastigote. Body pyriform or top-shaped; axostyle often projects in a point; radially symmetrical. Neuromotor system consists of about sixteen long anterior flagella, rhizoplast, centroblepharoplast, a stout, fibrous axostyle, and parabasal ring of eight rounded parabasal bodies. Centroblepharoplast has a flattened, deeply staining cap, which is not found in any of the related genera. Single anterior nucleus.

Type species.—*T. octoplus* from *Reticulitermes hesperus* Banks.

Torquenympha octoplus sp. nov.

Average size 11 by 20 microns; eight ovoid parabasal bodies in the parabasal ring; central axostyle which surrounds the nucleus and tapers posteriorly to a sharp point. Spirochaetes may be present attached to the posterior end.

Host.—*Reticulitermes hesperus* Banks from Berkeley, California.

Microjoenia pyriformis sp. nov.

Body shape pyriform; length 44–52 microns, width 24–30 microns. 18–20 anterior flagella attached to prominent longitudinal rows of basal granules or to the cap-like centroblepharoplast; 12–16 anterior longitudinal lines of basal granules. Axostyle narrow with few granules. Single anterior nucleus; endosome ellipsoidal and composed of dispersed chromatin granules.

Host.—*Reticulitermes hageni* Banks from Glenford, Georgia.

Microjoenia ratcliffei sp. nov.

A small xylophagous hypermastigote. Body shape pyriform; axostyle sharply pointed with an axial filament, anterior end flattened on one side; sixteen to twenty anterior flagella are attached to a blunt centroblepharoplast or to the 16 to 18 longitudinal rows of basal granules. These anterior rows of basal granules extend for one-fourth of the body length. Single anterior nucleus.

Host.—*Reticulitermes hesperus* Banks from Berkeley, California.

SUMMARY

1. A xylophagous flagellate, with an anterior parabasal ring, central axostyle, and single anterior nucleus, is described as a new genus, *Torquenympha*.

2. *Torquenympha octoplus* sp. nov. is described as a new species.

3. The neuromotor system consists of about sixteen anterior flagella, rhizoplast, centroblepharoplast, central axostyle, and a parabasal ring of eight ovoid parabasal bodies.

4. *Microjoenia* is considered to be a valid genus and not a part of the life-cycle of *Spirotrichonympha*.

5. *Microjoenia ratcliffei* sp. nov. and *Microjoenia pyriformis* sp. nov. are described as new species from *Reticulitermes hesperus* Banks and *Reticulitermes hageni* Banks respectively.

6. The parabasals of these flagellates cannot be considered as homologues of the Golgi apparatus.

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EXPLANATION OF PLATES

PLATE 4

All figures are camera lucida drawings of *Torquenympha octoplus* gen. nov., sp. nov., from *Reticulitermes hesperus* Banks. Figures 2, 3, and 5 are from intestinal smears fixed in Champy's fluid; figures 1, 6, 7, and 9 from material fixed in Flemming's fluid (without acetic); figures 4 and 8 from smears fixed in Schaudinn's fluid (with very little acetic). All were stained in iron-haematoxylin. × 1830.

Fig. 1. External or surface view showing six of the eight parabasals; axostyle is fibrous.

Fig. 2. An apical view showing all eight parabasals; numerous bacteria are attached.

Fig. 3. Note granular cytoplasm, sixteen flagella and the basal granules on the base of centroblepharoplast.

Fig. 4. Note that axostyle did not take a stain; nucleus is ovoid.

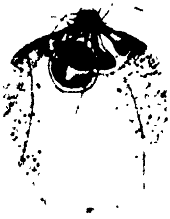
Fig. 5. Five of the eight parabasals are shown with ''myoneme-like'' strands attached to the base of the parabasal ring.

Fig. 6. Four of the eight parabasals are shown. Note that numerous bacteria are attached to the body.

Fig. 7. Axostyle is very faint, probably due to fixation.

Fig. 8. Nucleus is typical with a large endosome. An anterior view with all eight parabasals.

Fig. 9. Lateral view with one-half of the parabasals shown.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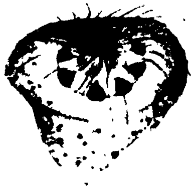
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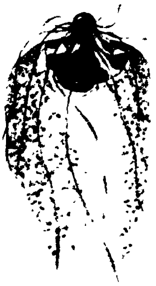
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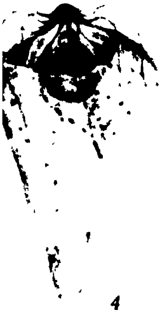
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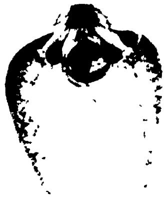
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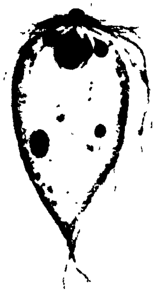
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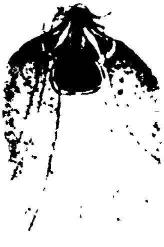
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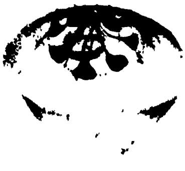
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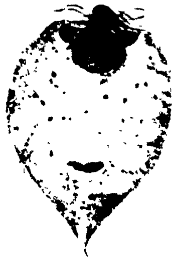
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PLATE 5

All figures were drawn with the aid of a camera lucida. Figures 11 to 16 are *Microjoenia pyriformis* from *Reticulitermes hageni* Banks. Figures 10, 12, 13, 14, 15 and 17 are *Microjoenia ratcliffei* from *Reticulitermes hesperus* Banks. Figures 10, 12, 14, and 15 are from slides fixed in Flemming's strong without acetic; figures 11, 13, 16, and 17 are from material fixed in Champy's solution. Heidenhain's iron-haematoxylin was used as a stain. × 1830.

Fig. 10. Anterior view of *Microjoenia ratcliffei* showing longitudinal lines with basal granules, central axostyle and large endosome.

Fig. 11. *Microjoenia pyriformis* with a stout axostyle, chromatin distributed and no large endosome.

Fig. 12. Dorsal view of *Microjoenia ratcliffei;* note two anterior parabasals, one on either side of the nucleus.

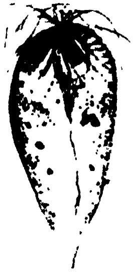
Fig. 13. Numerous mitochondria distributed throughout the cytoplasm.

Fig. 14. A lateral view of *Microjoenia ratcliffei;* note flattened ventral surface.

Fig. 15. *Microjoenia ratcliffei* with numerous longitudinal lines with basal granules.

Fig. 16. *Microjoenia pyriformis,* lateral view with heavy longitudinal lines. Body is slightly flattened on one side.

Fig. 17. *Microjoenia ratcliffei.* Golgi bodies are present as rings and spheres.



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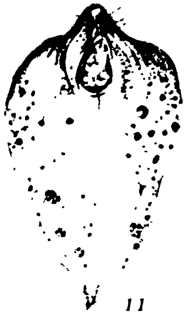

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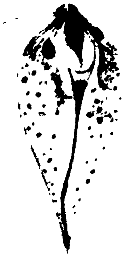

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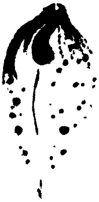

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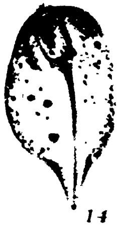

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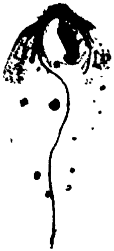

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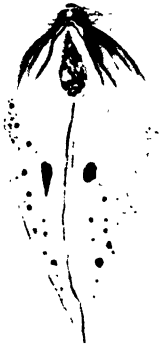

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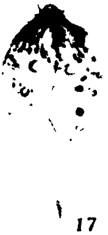

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THE EFFECT OF DIET UPON THE INTESTINAL FAUNA OF TERMOPSIS

BY

EVERETT EUGENE LUND

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THE EFFECT OF DIET UPON THE INTESTINAL FAUNA OF TERMOPSIS

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INTRODUCTION

That many termites live by ingesting only wood has long been considered merely a matter of observation. Among the genera which do this is *Termopsis*, the large rotten-wood termite common on the Pacific Coast. In this genus it will be remembered that the workers, which are the type most frequently chosen for experiments, do not constitute a distinct caste, but are merely immature forms (nymphs) of either soldiers or reproductive forms. These nymphs comprise by far the greater part of a colony at almost all times.

In the posterior part of the intestinal tract of *Termopsis* are found six species of Protozoa, four of which are well known from the work of Kofoid and Swezy (1919*a*, *b*, *c*, and *d*), Andrews (1925), and Kidder (1929), and two smaller forms, also flagellates, which have recently been described by Kirby (1930).

Cleveland (1925*a*) demonstrated the ability of *Termopsis* to live and maintain nearly its normal fauna almost indefinitely on a diet of pure cellulose. He also showed (1925*b*) that starvation in the same genus defaunated the termites in a fairly specific manner, removing the four species of larger Protozoa singly, and in quite a definite time.

Montalenti (1927) was able partly, and perhaps completely, to defaunate *Calotermes* and *Reticulitermes* by feeding them soluble carbohydrates.

The purpose of the experiments described in this paper is to determine how the Protozoa of *Termopsis* are affected when the termite is subjected to a diet of carbohydrates not containing cellulose, which is their normal food.

ACKNOWLEDGMENTS

I hereby acknowledge my indebtedness to Professor Charles A. Kofoid under whose supervision these experiments have been conducted, and to Dr. Harold Kirby and Dr. S. F. Light, whose suggestions have been of especial value.

MATERIALS AND METHODS

Knowing that the fauna of *Termopsis* can live almost indefinitely when the termite is fed higher carbohydrates, namely, the celluloses, it is of interest to see if they can also live, and if so, how well, on the lower carbohydrates, the starches and sugars.

The celluloses are complex polysaccharides having the general formula $(C_6H_{10}O_5)_n$, and are usually found in nature as compound celluloses made up of several isomers. Normal cellulose such as is found in cotton fibers and which is also present to quite an extent even in wood, is practically insoluble in water and organic solvents. It is rather inert generally, not being readily reactive at ordinary conditions with any of the common elements or compounds, except oxygen, and not subject to the action of any of the well-known enzymes in nature.

I have chosen to start my experiments with such a material, and to descend the carbohydrate series of polysaccharides, $(C_6H_{10}O_5)_n$; trisaccharides, $D_{18}H_{32}O_{16}$; disaccharides, $C_{12}H_{22}O_{11}$; and monosaccharides, $C_6H_{12}O_6$; using one or more representative substances of each group, each as the sole food of a number of termites during the course of the experiment.

For convenience, the substances used are listed below, with a brief review of their outstanding properties, and the number of the experiment in which each was fed. Wood and cellulose were used for controls.

POLYSACCHARIDES $(C_6H_{10}O_5)_n$. EXPERIMENTS I AND II.

AMYLUM (Starch).

Insoluble in cold water.
Granules burst in hot water, and opalescent solution is formed.
Hydrolysis to dextrines and maltose, and further to glucose.
Acted upon principally by diastase and ptyalin.

DEXTRIN. EXPERIMENT V.

Slowly soluble in cold water.

Readily soluble in hot water.

Hydrolysis to glucose by action of amylases.

INULIN. EXPERIMENT IV.

$(a)_D$ — 40°.

Practically insoluble in cold water, but soluble in warm.

Hydrolyzed by acids or inulase to fructose.

TRISACCHARIDES ($C_{18}H_{32}O_{16}$).

RAFFINOSE. EXPERIMENT XI.

$(a)_D + 104.5°$.

Not very soluble in water. 14 gm/100 c.c.

Reduced by invertase to fructose and melibiose, and by emulsin to sucrose and galactose.

DISACCHARIDES ($C_{12}H_{22}O_{11}$).

MALTOSE. EXPERIMENT XII.

$(a)_D + 137°$.

Very slowly soluble in cold water.

Hydrolyzed to glucose by enzyme maltase.

LACTOSE. EXPERIMENT XIII.

$(a)_D + 55.3°$.

Not very soluble in cold water. 17 gm/100 c.c.

Hydrolyzed to glucose and galactose by enzyme lactase.

SUCROSE. EXPERIMENTS XV AND XIX.

$(a)_D + 66.5°$.

Very soluble in water. 200 gm/100 c.c.

Hygroscopic.

Hydrolyzed to glucose and fructose by invertase (sucrase).

MONOSACCHARIDES.

HEXOSES ($C_6H_{12}O_6$).

LEVULOSE (FRUCTOSE). EXPERIMENT XVI.

$(a)_D$ — 93°.

Very soluble in water.

Very hygroscopic.

MANNOSE. EXPERIMENT XVII.

$(a)_D + 14.2°$.

Very soluble in water. 250 gm/100 c.c.

Hygroscopic.

DEXTROSE (GLUCOSE). EXPERIMENT XVIII.

$(a)_D + 52.5°$.

Moderately soluble in water. 83 gm/100 c.c.

GALACTOSE. EXPERIMENT XX.

$(a)_D + 80.3°$.

Very soluble in water.

Somewhat hygroscopic.

PENTOSES ($C_5H_{10}O_5$).

XYLOSE. EXPERIMENT XXI.

$(a)_D + 19°$.

Quite soluble in water. 117 gm/100 c.c.

Hygroscopic.

In a series of preliminary tests an effort was made to determine the best methods of feeding. In all cases fifty termites were used for each experiment, all nymphs of large and about equal size. Care was taken to select individuals that had neither moulted recently nor appeared to be ready to do so, in this way getting as nearly equal faunation as possible. The nymphs were all chosen from one colony which had had a normal wood diet up to the beginning of the experiment. Each group of fifty was placed in a five-inch finger bowl supplied with a one-inch Stender dish of water to keep the humidity as favorable as possible. (In some cases the water had to be removed because of the hygroscopic nature of the substance, as will be shown later.)

The following methods of introducing food material were tried:

I. The substance (.5 gm) was placed in the bowl in granular or crystalline form.
II. A solution or suspension of the substance in cold water was dried slowly in a thin film over the bottom of the bowl.
III. Asbestos, as a base, was saturated with the material described above (2), and dried.
IV. The substance in granular form was mixed with fine sand, and spread over the bottom of the bowl.

With the exception of method III, the results were almost identical. If the substance was hygroscopic a syrup was formed in any event, and if the termites encountered it they soon died. If it was not a hygroscopic substance, no advantage was exhibited by methods II or IV over method I. The use of asbestos as base was not satisfactory, for asbestos alone in a control group killed both Protozoa and termites in less than four weeks. Further investigation is necessary to explain this.

For the reasons given above, method I, or the feeding of crystalline or granular material in its usual chemical form, was used in all final tests except one of those in which starch was fed (Exp. I). There method II was used.

As before, fifty nymphs, selected in the manner explained above, were used; and the other material was identical to that described before. In Experiments XV, XVI, XVII, XIX, XX, and XXI, no water was left in the bowl as the sugars were quite hygroscopic, as has already been indicated. In some cases a few drops of water were added at intervals. Moreover, two groups were started for each substance, one group being used for frequent examination of living material, particularly during the first ten days, when changes were rapid, and the other group being used in making stained smears throughout the entire course of the experiment.

Dead termites were removed from the bowls as soon as their presence was noticed, in order to prevent the remaining ones from contaminating their diet by eating the dead. However, in many cases the pellets were not removed, and it seems probable that a little contamination might have been effected in some cases by the termites' eating these droppings.

As indicated before, during the first ten days of the experiment, observations of living material from the hind intestine of the termite were made daily. In all cases the intestine itself was removed in the usual way, broken, and its walls disturbed by a needle. (It is unsatisfactory merely to squeeze the abdomen of the termite, for *Streblomastix,* the most numerous of the four larger Protozoa, clings in vast numbers to the wall of the intestine. To get a fair estimate of the relative numbers of all the genera, these must be disturbed.) During the entire course of the experiment smears of the intestinal content were made at regular intervals, every second day during the first three weeks, and at intervals of three, four, and five days respectively over the time that remained. In some cases the termites might easily have outlived the time of the test had it not been necessary to remove two or three at the above-mentioned periods for examination. This indicates that larger groups might have been used advantageously. All smears were stained with iron-haematoxylin.

RESULTS

The results obtained were largely of a nature that can most easily be shown graphically. For this reason I have arranged a series of graphs, one for each experiment with significant results, and have supplemented the graphs with brief, individual explanations.

In general, the four major species of Protozoa, *Streblomastix strix, Trichomonas termopsidis, Trichonympha campanula,* and *Leidyopsis sphaerica,* are almost always present in termites on a normal wood diet in a fairly definite ratio. This I have found in my controls to be as follows, choosing the number of *Leidyopsis,* the least frequent, arbitrarily as 10:

Leidyopsis	10
Trichonympha	2,500
Trichomonas	6,500
Streblomastix	45,000

Of course, these proportions are found to vary in termites even under normal conditions, and they cannot be considered absolutely accurate for those used in getting the figures, as anyone who examines the congested mass of Protozoa in the intestine of a termite must realize. However, continued count gives proportions ranging about those mentioned above, providing the intestine is removed and its wall broken and disturbed to free the attached *Streblomastix.* Cleveland (1925, II) estimated the relative numbers of these four species quite differently, but I have not been able to confirm his estimate.

With these proportions all graphical representations of the numbers of the Protozoa on the successive days of each experiment start, and it is reasonable that any great and consistent variations from proportions may be attributed to the factors of the experiment, intentional or uncontrolled.

No effort was made even to estimate the numbers of *Tricercomitus termopsidis* and *Hexamastix termopsidis,* the two smaller flagellates, but their occurrence is mentioned in some of the experiments discussed below, if they seemed of particular significance.

Morphological changes in the Protozoa in most cases were either so slight or so varied as to be of no importance; or they were merely those that always accompany unfavorable conditions, and are familiar to everyone who has observed the intestinal fauna carefully. Exceptional tendencies are mentioned in the individual discussions of the experiments in which they occur.

EXPLANATION OF THE GRAPHS

In all cases, days of duration of the experiment are represented on the abscissa, numbering progressively to the right from the point *O, O.* A vertical line marks the death of the last termites, and if they were used for examinations this is indicated. The numbers of individuals of each species of Protozoa are represented on the ordinate, a logarithmic scale progressing upward from the point *O, O.* Owing to the great variation in the relative numbers of each of the Protoza, it was of course impractical to attempt to represent this factor on a simple arithmetical scale, so the logarithmic scale was selected because it accommodated the figures nicely and is not difficult to interpret. In this way the value numerically of any point can be found by determining its distance above the abscissa, which is its logarithm to base 10, and obtaining the cologarithm by means of a table or a slide rule.

Each centimeter along the ordinate represents a unit in the characteristic, and the fraction remaining, the mantissa.

It must be understood that results shown on the graphs are only approximate, and slight fluctuations are doubtless due to individual variation. In plotting them, the occurrence of *Streblomastix* was first determined for any day by comparing the results of a number of examinations made that day with those obtained in studying normal termites. Using this, then, as a standard, subsequent to the first day, the occurrence of the three other forms was plotted relative to it. In this way any error in estimating the frequency of *Streblomastix* becomes quite insignificant, numerically, when transmitted to the less numerous species.

INDIVIDUAL RESULTS OF THE FINAL EXPERIMENTS

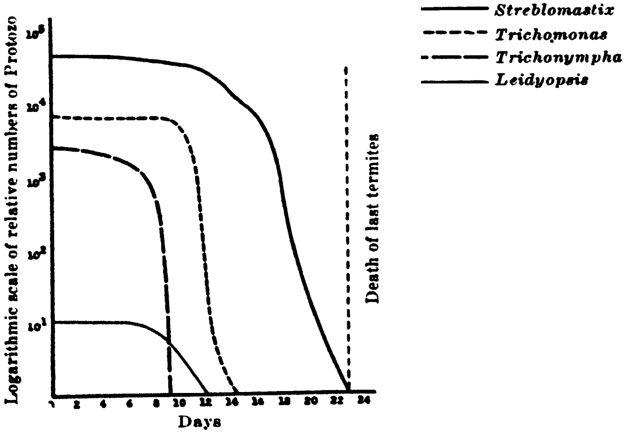
I. Amylum—

(Commercial cornstarch used.)

As the graph (fig. 1) for this experiment shows, death of both Protozoa and termites occurred early, after 23 days on the diet. The intestinal content when tested with iodine showed little free starch until the ninth day, by which time most of the Protozoa of greatest value to the termite were gone. Many of the remaining *Leidyopsis*, *Trichonympha*, and even *Trichomonas* had ingested granules of starch. *Streblomastix* was less frequently free in the intestinal fluid than

Figure 1

EXPERIMENT 1. AMYLUM



usual, but many were attached to the intestinal wall. *Tricercomitus* and *Hexamastix* were very numerous between the twelfth and twentieth days of the experiment.

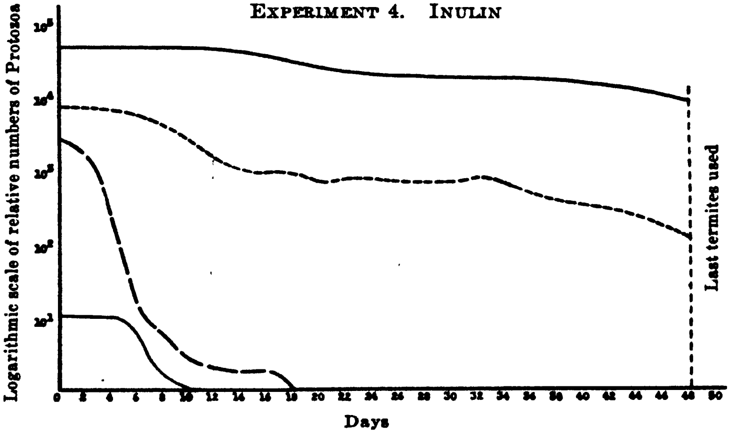
II. Amylum—

(In this case the starch had been pulverized in a crucible to break the granules.)

No graph appears for this experiment, for satisfactory estimates of the faunation were impossible. As soon as the termites were placed on the bowl they became covered with the finely powdered starch, groomed themselves and one another, and at once ingested great quantities of the material. Throughout the course of the experiment, 43 days, the intestine was filled with the thick, pasty solution, and counts could be made neither in fresh nor in stained material. *Trichonympha* and *Leidyopsis* were present as late as the fifteenth day, and may have occurred later. *Trichomonas* were found, especially very large rounded forms, until the fortieth day, and *Streblomastix* persisted until the death of the termites, the forty-third day, but rarely if ever occurred free in the intestine, but always clinging to the wall.

Figure 2

EXPERIMENT 4. INULIN



IV. Inulin—

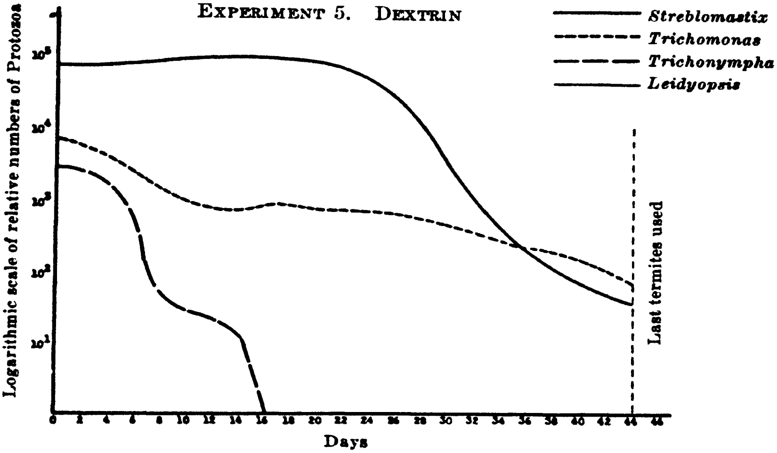
This experiment was ended by the necessity of taking the last termites for examination; it might have continued much longer. The graph (fig. 2) shows the fate of the four species of larger Protozoa. *Tricercomitus* and *Hexamastix* were exceedingly numerous during the latter part of the test.

V. Dextrin—

The results here (fig. 3) were quite similar to those of Experiment IV. However, *Streblomastix* declined rapidly in numbers after the twenty-second day; and during this decline many of the long whip-forms and giants, some measuring 350 to 400 microns, which Kidder (1929) described, were found. *Leidyopsis* was never observed, although it was probably present.

Figure 3

Experiment 5. Dextrin

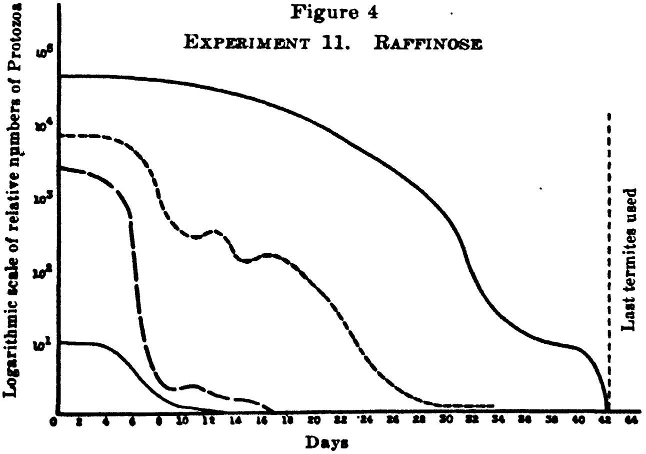


XI. Raffinose—

These results except as shown by the accompanying graph (fig. 4) differed in no important respect from those mentioned above for Experiment V.

Figure 4

Experiment 11. Raffinose



XII. Maltose—

In this test the termites were quite active at all times, the experiment ending as shown (fig. 5), by using the last individuals for examination. Even near the close of the test the Protozoa showed no consistent tendencies toward morphological changes.

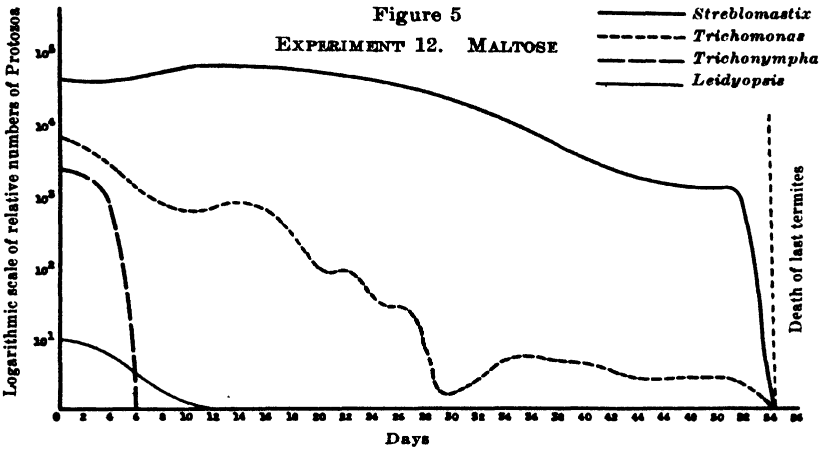


Figure 5
EXPERIMENT 12. MALTOSE

XIII. Lactose—

Results with the group of termites used in this experiment were the most successful of all (fig. 6). One termite was still alive and very active at the end of 65 days, and probably still contained Protozoa. In this test alone *Leidyopsis* was found throughout the period of examination. It was very rare in any individual, but many of them contained one or more specimens.

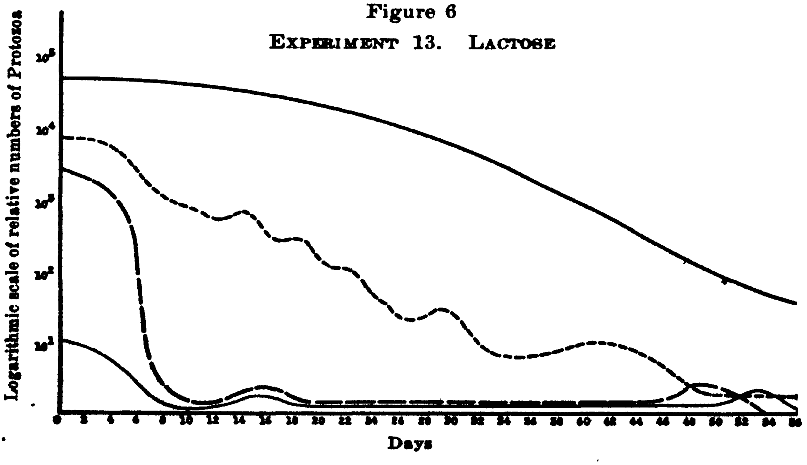


Figure 6
EXPERIMENT 13. LACTOSE

XV. Sucrose (Saccharose)—

One peculiarity in this experiment is worthy of mention. After the sixth day *Trichonympha* was more numerous than *Trichomonas,* and persisted longer, but the forms were rare and quite degenerate. In this group for the first time *Tricercomitus* and *Hexamastix* seemed to become less numerous during the course of the experiment. Moreover, giant and whip-like forms of *Streblomastix* were exceedingly rare, and usually not present at all (fig. 7). (These conditions occurred quite regularly in the tests that follow, and will therefore not be mentioned again except as a great variation appears.)

Figure 7

EXPERIMENT 15. SUCROSE

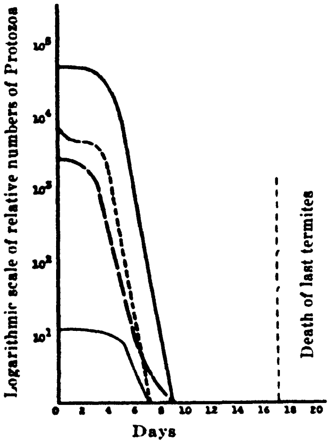


Figure 8

EXPERIMENT 16. LEVULOSE

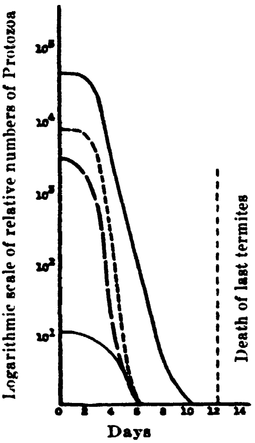
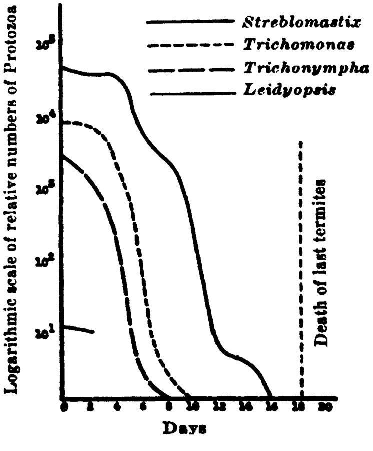


Figure 9

EXPERIMENT 17. MANNOSE



XVI. Levulose—

No results were observed beyond those shown in the graph (fig. 8).

XVII. Mannose—

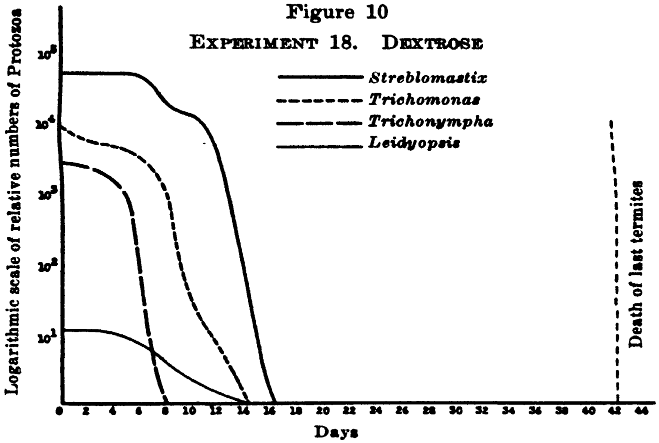
Leidyopsis could not be found after the second day, but this does not entirely justify a belief that it was absent (fig. 9).

XVIII. Dextrose—

After the disappearance of *Streblomastix* on the sixteenth day, *Tricercomitus* and *Hexamastix* appeared still to be present in moderate numbers up to the death of the termites. Bacteria, incidentally, became very plentiful in the latter half of the test (fig. 10).

Figure 10

EXPERIMENT 18. DEXTROSE

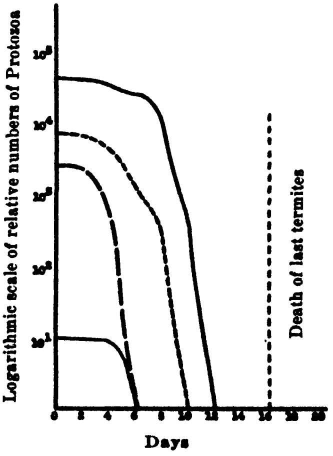


XIX. Sucrose (Saccharose, in rather large lumps)—

These results should check with those of Experiment XV. There is a discrepancy, but it is probably not beyond the variation occurring because of uncontrollable factors (fig. 11).

Figure 11

EXPERIMENT 19. SACCHAROSE



XX. Galactose—

Only the results indicated on the graph (fig. 12) were noted.

XXI. Xylose—

No results were observed other than those shown on the graph (fig. 13). Xylose, because of its great hygroscopic tendency, seemed to be entirely unsuited to the methods employed in feeding it.

Figure 12

Experiment 20. Galactose

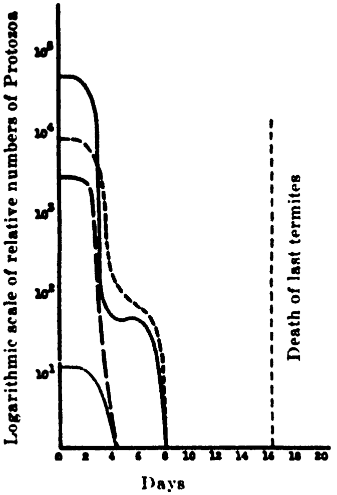
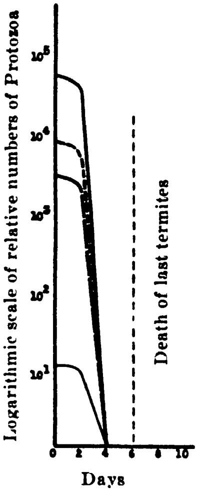


Figure 13

Experiment 21. Xylose



XXII. Starvation—

Although starvation tests were run a number of times, it will be sufficient here to give only one set of results, as they were reasonably consistent. The effect of such a test on the four species of larger Protozoa is shown in figure 14. During the last fourteen days of the experiment *Streblomastix* had a tendency to appear quite frequently in the long whip-like form. *Tricercomitus* and *Hexamastix* were abundant during much of this period, and persisted as long as the termites lived. As a whole, the results were not unlike those obtained by Cleveland (1925), and had care been taken to remove the droppings more frequently they might have coincided quite closely. They are, however, accurate enough for their purpose in this experiment.

SUMMARY AND CONCLUSIONS

Termopsis, whose normal food is wood, and which can live readily on pure cellulose, was kept on diets of various lower carbohydrates. The substances used were selected from the descending series of carbohydrate compounds, and included polysaccharides, trisaccharides, disaccharides, and monosaccharides, with a pentose as the lowest form used. The effect of such diets on the intestinal fauna of the termite was observed.

1. The relative numbers of the four species of larger Protozoa of *Termopsis* on a normal wood diet are about these:

Leidyopsis (arbitrarily chosen)	10
Trichonympha	2,500
Trichomonas	6,500
Streblomastix	45,000

but *Streblomastix* clings to the intestinal wall in vast numbers, and does not appear to be so numerous as indicated above unless the wall is disturbed.

2. These proportions are varied by changing only the diet of the termites.

3. Different carbohydrate diets seem to have little morphological effect upon the major intestinal fauna of *Termopsis,* except those that always accompany adverse conditions, and they are undoubtedly signs of degeneration. The most outstanding examples of this were the appearance of giants, or whip-like forms of *Streblomastix,* and of very large, almost motionless *Trichomonas,* both of which occurred only late in the experiments.

4. In the experiments in which the higher carbohydrates, trisaccharides to starches, were used, the Protozoa undoubtedly were able to assimilate some of the material. They were observed to ingest it, and they were usually present from 50 per cent to 200 per cent longer than they were in starved termites. In general, the results seemed to indicate that the process was one of prolonged starvation.

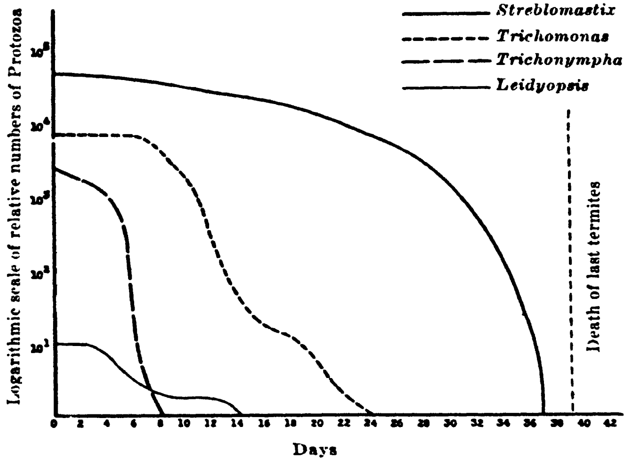
5. Starch dried in thin sheets and used for feeding does not give significant results, for the termites do not eat of it until they are very hungry, and by that time the greater part of their most valuable Protozoa have died of starvation.

Pulverized starch is equally unsuitable in such a test, for great quantities of it are ingested in grooming, and the intestinal fluid is not suitable as a medium for the Protozoa. This is evidenced by the migration of *Streblomastix* to the walls of the intestine.

6. In the experiments in which the soluble materials were used, all disaccharides and monosaccharides, the Protozoa died very quickly; more quickly than on the less soluble higher sugars, and the starches. This is due to a combination of three things. When very soluble sugars are ingested they go at once into solution in the intestinal fluid. This changes the osmotic pressure, probably beyond the range favorable to the Protozoa. Moreover, with the exception of *Streblomastix*, which appears to be a true parasite rather than a symbiont, the major Protozoa of *Termopsis* are holozoic; and if all the food is in solution they are not able to ingest enough to keep alive. Then too, the more soluble substances are, in general, more hygroscopic, and this makes the humidity of the chamber difficult to control. Extreme variations of this factor reduce the faunation of *Termopsis* surprisingly.

Figure 14

EXPERIMENT 22. STARVATION



Of these three conditions, the changing of the osmotic pressure is the most important, as is evidenced by Experiment XVIII, where a moderately soluble, but non-hygroscopic substance was used, and by the fact that *Streblomastix*, which is probably not holozoic, perishes with the rest when fed soluble substances.

7. The humidity has a profound effect upon both termites and Protozoa; and better methods of controlling it during the course of

experiments such as were conducted would make the results easier to interpret.

8. The dextro- and laevo-rotary properties of the various sugars seemed to have no effect on any of the Protozoa.

9. The results give no satisfactory clues to the enzymes present in either the Protozoa or the termite.

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Transmitted July 16, 1930.

NOTES ON THE GENUS ENDOSPHAERA ENGELMANN AND ON ITS OCCASIONAL HOST OPISTHONECTA HENNEGUYI FAURÉ-FREMIET

BY

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INTRODUCTION

The genus *Endosphaera* was established by Engelmann (1876) for the endoparasitic Suctoria without tentacles, which parasitize the Vorticellidae. Engelmann published no detailed description of the genus, and named no species, but he proposed the generic name *Endosphaera* and described and figured the genus more or less adequately in various papers on the Infusoria, in particular in those papers dealing with "embryo formation" in ciliates (1862, 1876).

The protozoologists of the middle of the last century mistook the various endoparasitic Suctoria which they observed in the ciliated Infusoria for embryos of the host organism. This "embryo hypothesis" had many supporters, including the great student of the Protozoa, Friedrich von Stein (1867). Balbiani (1860) seems to have been the first to combat this embryo theory and to advance the "parasite hypothesis," which stated that the so-called embryos of the ciliates (Suctoria excluded) actually were parasitic Suctoria of the genus *Sphaerophrya*.

Engelmann at first (1862) was an advocate of the embryo hypothesis. Later (1876), he became a protagonist of the parasite hypothesis, and at that time proposed the generic name *Endosphaera* to receive the Suctoria, entozoic in the Vorticellidae, which have no tentacles at any time in their life cycle, and which differ from the genus *Sphaerophrya*, whose embryos possess tentacles.

G. Entz, Sr. (1876) lists the genus *Endosphaera* in his Fauna of Hungary and proposes the specific name *engelmanni*, without, however, giving any description or figures of the species. The species, *Endosphaera engelmanni,* has since been listed in various keys, but to date, to the best knowledge of the authors, the species has never been adequately described.

The writers have been fortunate in encountering this protozoan in great abundance, and in being able to observe practically all the stages in its life cycle. They propose, therefore, to publish a description with measurements, which will define the species *Endosphaera engelmanni* accurately enough to serve as a basis for distinguishing other species of the genus, if such occur. Since the *Endosphaera* which they observed was parasitic in the rare vorticellid, *Opisthonecta henneguyi,* they will add a brief description of this species, thus contributing a few new details to the excellent description of Fauré-Fremiet (1923).

Opisthonecta henneguyi was found in great abundance in the pond in the tree-fern glen in Golden Gate Park, San Francisco, on December 16, 1928. The pond was about 50 feet wide and 150 feet long, and was covered with a sheet of ice a quarter of an inch thick, which persisted throughout the week in which collections were made. The pond is fed by two small streams which come from the septic tanks of cesspools. During the rainy season the run-off from adjacent slopes helps to fill the pond, which consequently fluctuates much in size. Collections were made on December 16, 17, 19, 21, 24, and 26. The organisms were less and less abundant at each visit and were entirely absent on December 26.

Associated with *Opisthonecta henneguyi* were the following forms: *Trachelomonas crebea, Didinium nasutum, Paramecium multimicronucleata, Spirostomum teres, Carchesium* sp. (one colony, only, was seen), *Hydatina senta, Cyclops sp.*

THE HOST

The host of *Endosphaera,* in this case, is *Opisthonecta henneguyi* Fauré-Fremiet (1923), a free-swimming vorticellid which lacks any trace of a stalk or scopula. It has the shape of a truncated cone with a rounded base. At the junction of the rounded base with the body of the cone is an annular welt or ridge bearing the long locomotor cilia which average 22.5 (20 to 26) μ in length. At the oral end, just beneath the epistomal disc, is another annular welt or collarette containing several sphincter myonemes, which contract the borders of the collarette over the epistomal disc when it is withdrawn.

The *adoral cilia* occur in two parallel rows bordering a shallow groove, and form a spiral of about 1.1 turns. The inner row of the adoral spiral is composed of a heavy undulating membrane, frayed at the outer border. The outer row is much less easy to resolve, but it also is probably composed of a membrane fimbriated along the margin. The outer membrane apparently stops at the mouth, or opening into the vestibule; the inner membrane continues down the length of the vestibule in a spiral path. The adoral ciliary spiral is borne on the projecting epistomal disc characteristic of the Vorticellidae. The inner membrane is 8–9μ high, the outer one, 10μ in height. Just above the opening into the vestibule is a conspicuous papilla bearing about a dozen very long, delicate, immobile cilia or bristles 23–27μ in length (''flamme épistomienne'' of Fauré-Fremiet).

The length of freshly collected specimens of *Opisthonecta* ranged from 148 to 163μ. After the collection had stood in the laboratory for several days the specimens became very much smaller, down to a length of 115μ. Their width was extremely variable, amounting in the region of the locomotor cilia to from 63 per cent to 85 per cent of the length. The epistomal disc was about 65 per cent the width of the widest part of the body.

Epistomal retractor myonemes can be seen in the living animal (pl. 6, *ret. my.*) running from near the apex of the epistomal disc about half the length of the body toward the aboral end. They are broadest at the apical end, and taper as they run aborally. In the last 20–30μ of their length they branch once or twice. In the region of the collarette the retractor myonemes are spaced about 10μ apart. Some have inconspicuous branches in the region of the collarette, the branches probably being attached to the ciliated adoral spiral, although their precise terminations could not be made out.

The *sphincter myonemes* within the collarette consist of a large, heavy, annular myoneme beneath which, i.e., aborally, are three others less than half the thickness of the first one. In fixed and sectioned material these four rings often seem to anastomose (fig. A, *sph. my.*). This is possibly an artifact, since no anastomosis or crossing of the myonemes was observed in living individuals. In material sectioned and stained with iron-alum haematoxylin the collarette is seen to be provided with numerous fine radially arranged striae, which undoubtedly are retractor myonemes of the collarette.

Surface ridges of extreme delicacy run circularly (transversely) around the body from end to end. Their presence on the epistomal disc was not noted. Ten of these transverse surface markings occur in a space of 5μ. They can be seen in live specimens, and are distinctly demonstrated by the Bresslau relief staining method, with opal blue, but are not to be seen after ordinary fixing and staining.

The *locomotor cilia* arise from an annular band 1.5μ wide near the posterior border of the annular ridge upon which the band is situated. Aborally this band is bordered by a delicate ridge 0.5μ wide, orally it is bounded by a very delicate fiber. Between the two fibers are conspicuous oblique striations 0.5μ apart, which probably correspond to the bases of membranelles or cilia of the locomotor girdle. From these oblique striations delicate longitudinal fibrils, likewise spaced 0.5μ apart near their origin, run to the aboral pole, just beneath the circular, or transverse, surface markings.

Fauré-Fremiet describes the locomotor ciliary band as "constituée par une rangée annulaire de membranelles surmontée d'une membrane ondulante." The writers were not able to determine with certainty whether or not these membranelles are united into a membrane. The usual impression they made was that of long and extremely delicate cilia. However, from the width of their bases they certainly must correspond to membranelles, and if they are united to form a membrane, which is unlikely, the membrane is certainly fimbriated at its margin.

In live animals there can be seen just beneath the pellicle large numbers of rather evenly spaced, colorless granules, circular, rod-shaped, or irregular in outline. These granules are not seen in whole mounts fixed with routine fixing fluids, but in material fixed in Flemming's fluid without acetic, and stained with iron-alum haematoxylin, they are preserved and conspicuous (fig. A, *mit.*). They tend to be circular in outline in the region of the epistomal disc, from 0.5–1μ in diameter. In other parts of the body they are, for the most part, rod-like, or dumb-bell shaped. The elongated types vary from 1–3μ in length, and from a fraction of a micron to one micron in width. These structures undoubtedly are mitochondria, although the writers undertook no specific techniques to demonstrate their nature. They are most abundant just beneath the pellicle, but occur more sparsely throughout the cytoplasm.

The *cytoplasm* of *O. henneguyi* is very finely granular and very transparent. There is no distinct ectoplasm. There is always a conspicuous aggregation of coarse granules in the aboral pole.

The *meganucleus* is extremely variable as to size and shape. In a large proportion of the *Opisthonecta* the *meganucleus* is a simple sausage-shaped organ; i.e., an elongated cylinder bent around to form almost a circle, and is located near the anterior end. It may lie in any plane, but usually lies more or less parallel with the surface of the expanded epistomal disc. However, the meganucleus is often much longer and may stretch almost from end to end of the animal. It often has extremely convoluted shapes such as figures S, spirals, or may even be tied into knots. In fact, in a large proportion of the *Opisthonecta* the meganucleus is so very long, and so inordinately and intricately twisted and intertwined as to defy tracing and measurement. The length varies from 60μ to more than 165μ, the breadth from 5–20μ. The meganuclei of nearly all individuals contain scattered globules looking like oil, and ring and ring-within-ring structures as if hollow bubbles occurred within the nucleus. The globules range from very minute sizes to 10μ in diameter.

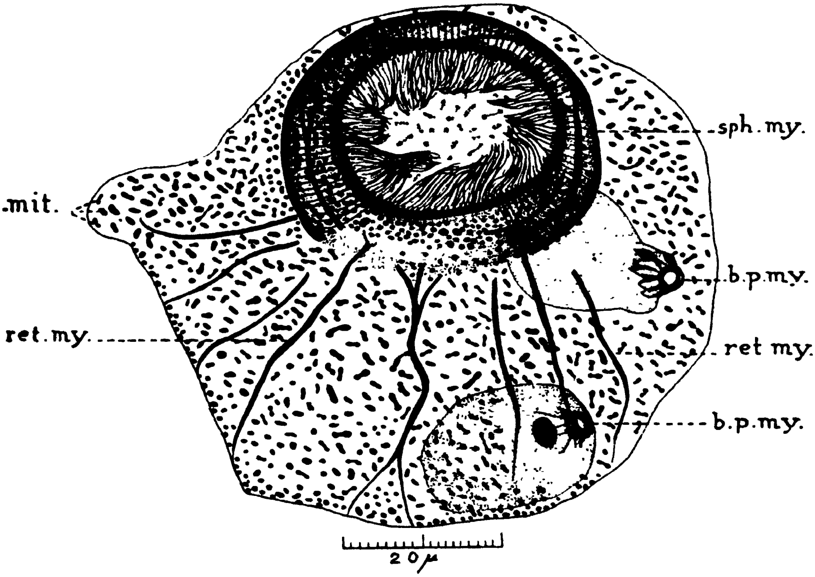

Fig. A. Slightly oblique section of the anterior end of *Opisthonecta*, with portions of two *Endosphaera* in the section. Flemming's fluid without acetic acid. Iron-alum haematoxylin. × 1025. Abbreviations: *b. p. my.*, birth pore of *Endosphaera*, with radial myonemes; *mit.*, mitochondria; *ret. my.*, retractor myonemes of epistomal disc; *sph. my.*, sphincter myonemes of collarette.

The *micronucleus* could not be seen in the live animal and it is not mentioned or figured by Fauré-Fremiet. In fixed specimens, however, stained with borax carmine or by Feulgen's nucleal reaction the micronucleus can be seen (pl. 6, *mic.*). It presents a mass of chromatin of spindle shape averaging 6 by 2 (4.5 to 8 by 1.5 to 3) μ within a membrane averaging 8 by 4 (7 to 12 by 3 to 5.6) μ. The micronucleus is always situated near the oral pole of the animal.

The *vestibule* or *pharynx* slopes dorsally from its entrance at a gentle angle and in most individuals turns more or less to the right (if we consider the epistomal end as anterior, with the "mouth" on the ventral side of the anterior end). In a few individuals, however, the pharynx does not curve to the right at all, but continues in a rather straight course or may even swerve to the left. The capacious pharynx terminates in a long narrow esophagus which opens into the cytoplasm in the posterior one-third of the organism.

The *inner adoral membrane* continues into the pharynx as a spiral membrane. Seen in optical section it gives the impression of a number of large, powerful membranelles in the pharynx.

Three *contractile vacuoles* (pl. 6, *c. v.*) emptying into the pharynx seem constant, but occasionally four are seen. The position of these vacuoles in relation to the main axis of the pharynx is variable, but in general their location is as follows: there is one large vacuole at the oral end, typically ventral and to the right of the pharynx; a smaller one about two-thirds of the distance toward the aboral end, ventral and toward the left; and slightly aboral to the second one a third, dorsal and to the right. In addition to these three, in occasional individuals a fourth is seen, near the middle of the pharynx. A cytoproct occurs in the anterior third of the pharynx in the region indicated in the figure (pl. 6, *cyt.*). Food consists only of the most minute particles, apparently bacteria.

Opisthonecta is almost continuously in motion, although it is a slow and ineffective swimmer. Its motion is retrograde; i.e., it swims aboral end forward, and revolves clockwise as seen from the oral end. It moves in a close spiral, the aboral end describing larger circles than the oral end. These circles at times become so large that the animal merely "loops the loop" without progressing. When describing such circles the animal turns toward the ventral aboral "corner"; i.e., toward the point on the aboral end corresponding to the position of the mouth on the oral end. Often it rests on the substratum, on either the oral or aboral pole, and rotates for a time in one place while describing very small circles.

THE PARASITE

The *adults* of the *parasites, Endosphaera engelmanni,* appear as spheroidal bodies each with a conspicuous nucleus, located in the anterior (oral) half of the *Opisthonecta,* usually just beneath the surface of the host. In the living organisms no connection of the parasite with the pellicle of the host was noticed, although such a connection exists, as will be shown later. The number of parasites per host, as determined from the examination of one hundred fixed and stained *Opisthonecta,* varied from none to 12, and averaged 3.06. Ninety-seven per cent of the *Opisthonecta* were infected. In the living condition the parasites have a faint bluish, or blue-green tint as seen by transmitted light, which causes them to stand out conspicuously against the whitish, or yellowish cytoplasm of the host. In the live *Endosphaera* the cytoplasm appears homogeneous and glassy.

The *meganucleus* (pl. 7, fig. 9, *meg.*), with a dry objective, gives the appearance of being crowded with coarse granules about 1μ in diameter. When studied with high-power immersion lenses, and careful focusing, the meganuclei give the impression of being filled with a highly convoluted thread of coarse dimensions whose bends and twists look like granules as seen in optical plane or with low magnifications.

The *micronucleus* (pl. 7, fig. 9, *mic.*) could not be detected in the live parasite. In fixed and stained material it has the appearance of a dense spherical mass of chromatin 2μ in diameter, shrunken within a delicate membrane which forms a vesicle 3–4μ in diameter about the chromatin.

One *contractile vacuole* (pl. 7, fig. 4, *c. v.*) is present, typically located on the side nearest the surface of the host; or, if an embryo is present, somewhere near the border of the embryo. In normal specimens the contractile vacuole does not exceed five microns in diameter.

The diameter of the parasites showing no trace of embryos averages 21 (15–32) μ. The outline is circular or very slightly oval (disregarding the "birth canal" which is not seen in live specimens). The nucleus is 9–17μ in diameter, from 40 per cent to 56 per cent of the diameter of the cell, circular to oval in outline.

Parasites with embryos average 34 (33–41) μ in diameter. Their outline is circular to slightly oval. The nuclear outline of embryo-bearing parasites is circular, oval, reniform, or rarely of various odd or asymmetrical shapes. Usually it is crescentic or transversely elongated as if compressed by the spherical embryo on one side of it. The nucleus of such parasites varies from 10 × 16 to 15 × 19μ in length and depth.

The embryos within the parent average 14 (12–17) μ in diameter, and are spherical or slightly ovoid in shape. The meganucleus is spherical, 5.5 to 8μ in diameter. The micronucleus is invisible in live embryos. In fixed and stained material it is evident as a dense spheroidal mass of chromatin 1μ in diameter within a delicate membrane 2–3μ in diameter. The membrane and space surrounding the chromatin mass were best seen following the Feulgen method; after other methods usually the chromatin mass alone was visible. A contractile vacuole is present, 3μ in diameter when distended. Embryo formation is endogenous. In young stages the embryo can be observed within the parent, sharply outlined, but not within an obvious chamber. In older stages the embryos lie within a brood chamber (pl. 6, *End. emb.;* pl. 8, figs. 13, 14, 15) and the beating of the cilia and rotation of the embryo within the chamber can be seen.

The great majority of *Endosphaera* contain only one embryo, but occasionally one is seen with two, and in one instance one was observed with three.

The parasites are attached to the pellicle of the host by a short stalk perforated by a canal terminating in a birth pore. As previously mentioned, the writers failed to observed this canal in the living animals, and had the impression that the parasites were simply suspended in the protoplasm of the host. The first indication that such was not the case came from Mr. M. H. Simmers, teaching fellow in the Department of Zoology, University of California, who dissected an *Opisthonecta* with a micro-manipulator, and informed the writers that *Endosphaera* was attached to the host pellicle by a "stalk." Unfortunately, this occurred just at the time that *Opisthonecta* had disappeared from the pool, and it was impossible to get more material with which to repeat the experiment.

A careful study of whole mounts and sections gradually convinced the writers that *Endosphaera* does indeed remain in connection with the pellicle of the host by a short canal reminding one of the neck of a jug. In whole mounts the connection is usually inconspicuous and can only be seen in those individuals so situated that the canal appears in profile; i.e., on the border of the parasite, between it and the pellicle of the host. In sections of material fixed in Flemming's fluid and stained in iron-alum haematoxylin the canal is often conspicuous and deeply stained (pl. 7, fig. 8; pl. 8, fig. 11; fig. A).

The canal has the function of serving as a place for the egress of the mature embryos, and, judging from the location of the contractile vacuole the canal may also serve to carry off the waste products of the parasite to the surrounding medium. So in spite of the fact that it serves as an organ of attachment, there seems to be no reason for attempting to homologize it with the peduncle of the free-living stalked Suctoria. It is obviously the homologue of the birth pore of such a suctorian as *Tokophyra quadripartita.*

In all the preparations in which it can be observed, it has the structure of a short canal, 2–4μ in length by 2–3μ in breadth. At its attachment to the host pellicle there is a heavy annulus (fig. A, *b. p. my.;* pl. 7, figs. 8, 10; pl. 8, fig. 11) often staining deeply with iron-alum haematoxylin following fixation in Flemming's fluid. The canal itself has thickened walls, and frequently there is an appearance of additional delicate rings below the one at the surface. A certain proportion of the specimens also exhibit radial or longitudinal striations, or a fretwork with radially elongated meshes (fig. A, *b. p. my.;* pl. 8, figs. 11, 13) which may be confined to the neck or which may extend over the walls of the brood chamber (pl. 8, fig. 13). It is quite probable that these circular and longitudinal striae are delicate myonemes which open and close the birth pore.

The *embryos* are formed just beneath the birth canal within the pellicle of the parent. As the embryo matures it finally comes to lie within a brood chamber large enough to permit of ciliary movements and rotation. When the embryo is discharged it seemingly ruptures the brood chamber and is forced out of the now dilated birth pore. The enlargement of the birth pore that takes place during extrusion of an embryo possibly is brought about by the contraction of radial myonemes. The cytoplasm of the parent follows the embryo through the birth pore, appearing as a nose-like projection beyond the surface of the host, to which the embyro adheres a few moments before finally swimming off (pl. 6, *dis. emb.*; pl. 8, fig. 16).

Although the writers observed the extrusion of embryos repeatedly when surveying with low magnification dishes containing living *Opisthonecta,* they were unable to discover and follow through the process with high magnification. However, the final stage, with the extruded embryo adhering to the projection of the parent, was observed several times under high magnification, and various stages of the process have been found, perfectly preserved, in fixed material (pl. 8, figs. 14, 15, 16).

The fate of parent parasites which have discharged their embryos was not ascertained, but since no degenerating parents were seen, it seems probable that the protoplasmic projection is withdrawn, the birth pore contracts, and that the parent then proceeds to the formation of a new embryo.

The *free-swimming ciliated embryos* are practically spherical in shape, 15.7 (13.2–18.75) μ in diameter. They have three equatorial bands of cilia, the length of the cilia being half, or a little more than half, the diameter of the embryo. As seen from the pole, the cilia all curve in one direction (pl. 6, *dis. emb.*). There is a contractile vacuole located laterally at the level, or a little posterior to the level of the rows of cilia. This vacuole attains a maximum diameter of about 4μ in normal individuals. Often two small accessory vacuoles can be seen, one anterior and one posterior to the main vacuole. The meganucleus in the motile stage is 8μ in diameter, and is located somewhat laterally in the region of the equator of the embryo.

The ciliated embryos swim rapidly with an oscillating movement, owing to their swimming in a close spiral. They sometimes stop and spin around as if being whirled at the end of a string of short radius. Often they stop and stand on the substratum on either pole, and rotate rapidly with an oscillating movement, as though the axis were off center in the region of the contractile vacuole.

The ciliated embryos were repeatedly observed by both authors to attach themselves to various *Opisthonecta* and to adhere for long periods of time, but the writers were never able to follow through complete penetration in living material. It is probable that the process of penetration proceeds at a very slow rate. On the other hand, fixed and stained material shows the successive stages of penetration (pl 7, figs. 3–7). The evidence from these penetration stages in fixed material is that the free-swimming larva, after attaching itself to the pellicle of the host, produces a small perforation in the pellicle, and then squeezes in through this opening. There is no evidence that the pellicle of the host is pushed ahead of the invading parasite, thus eventually forming a chamber invaginated from the surface.

Encystment of *Opisthonecta* occurs readily, as Fauré-Fremiet states, the cysts being spherical or slightly ellipsoid, thick-walled and without any sculpturing or projections. The cysts vary from 65–77μ in diameter. The cyst wall is 5–6μ thick, consisting of an inner layer about 5μ thick, grayish or colorless, surrounded by an outer membrane 1–1.5μ thick, bluish by transmitted light. The senior author, who alone observed the process of encystment in *Opisthonecta,* noted that the encysted *Opisthonecta* contained *Endosphaera.* In one case only, excystment was observed with the microscope, and the *Opisthonecta* which emerged from the cyst contained two parasitic *Endosphaera.* The writers regret that fixed material of cysts of *Opisthonecta* containing parasitic *Endosphaera* was not prepared. The evidence from these observations is that *Endosphaera engelmanni* has no free-living, stalked stage, and no independent encysted stage, but that it tides over periods of unfavorable conditions within the cysts of its hosts.

It should be remarked, however, that during the week of April 21 to 28, 1929, the pool where *Opisthonecta* originally was found was again swarming with the organism, but in this case there was no sign of parasitism by *Endosphaera.* This is very curious, indeed, in view of the almost 100 per cent infestation of the *Opisthonecta* during the previous swarming.

DIAGNOSES

Since all the authors who have mentioned or described *Endosphaera engelmanni* Entz have published descriptions of the briefest kind, without measurements, the writers of this paper will attempt to give an adequate generic and specific diagnosis of the form.

Genus **Endosphaera** Engelmann, 1876

Small internal parasites belonging to the family Acinetidae; body spherical, without tentacles at any stage in the life-history; no test, cup, or stalk; reproduction by the formation of endogenous embryos; free-swimming embryo with three equatorial bands of cilia.

Endosphaera engelmanni G. Entz, 1896

Parasitic stage spherical, 15–41μ in diameter, imbedded in cytoplasm of host, but attached to the pellicle of the host by a short birth canal; free-swimming embryo spherical, 13–19μ in diameter; with three equatorial bands of cilia; both parasitic and free-swimming stages with one spherical meganucleus, one micronucleus, and one contractile vacuole; parasitic in the Vorticellidae (see Sand [1899] for list of known hosts).

SUMMARY

This paper describes the parasitic suctorian *Endosphaera engelmanni* Entz 1896 and one of its hosts, *Opisthonecta henneguyi* Fauré-Fremiet 1923. The description of *Opisthonecta* differs from that of Fauré-Fremiet in a few supplementary details. The parasitic and free-swimming stages of *Endosphaera engelmanni* are described in detail, with measurements. The penetration of the parasite into the host, the attachment of the parasite to the host pellicle, the strucure of the birth pore, and the discharge of the free-swimming embryos are described and figured. Evidence is presented that *Endosphaera engelmanni* has no independent encysted stage, but that it tides over unfavorable conditions within the cysts of its host.

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EXPLANATION OF PLATES

Plates 7 and 8 illustrate the life-history of *Endosphaera engelmanni* as interpreted from microscopic preparations. All figures of plates 7 and 8 are drawings either of optical sections of whole mounts, or actual sections, as noted below. The drawings were made with a Zeiss Zeichenprisma, and reduced one-third in reproduction. All the figures are drawn at the same scale, and appear at a magnification of 800 diameters in the plates.

PLATE 6

Opisthonecta henneguyi, parasitized by *Endosphaera engelmanni.* Freehand drawing from living material. × 750. Abbreviations: *c. v.*, contractile vacuole; *cyt.*, cytoproct; *dis. emb.*, embryo of *Endosphaera* being discharged through birth pore at surface of host; *End.*, *Endosphaera; End. emb.*, *Endosphaera* containing an embryo; *f. s. emb.*, free-swimming embryo of *Endosphaera; meg.*, meganucleus of *Opisthonecta; mic.*, micronucleus of *Opisthonecta; ret. my.*, retractor myonemes of epistomal disc; *sph. my.*, sphincter myonemes of collarette.

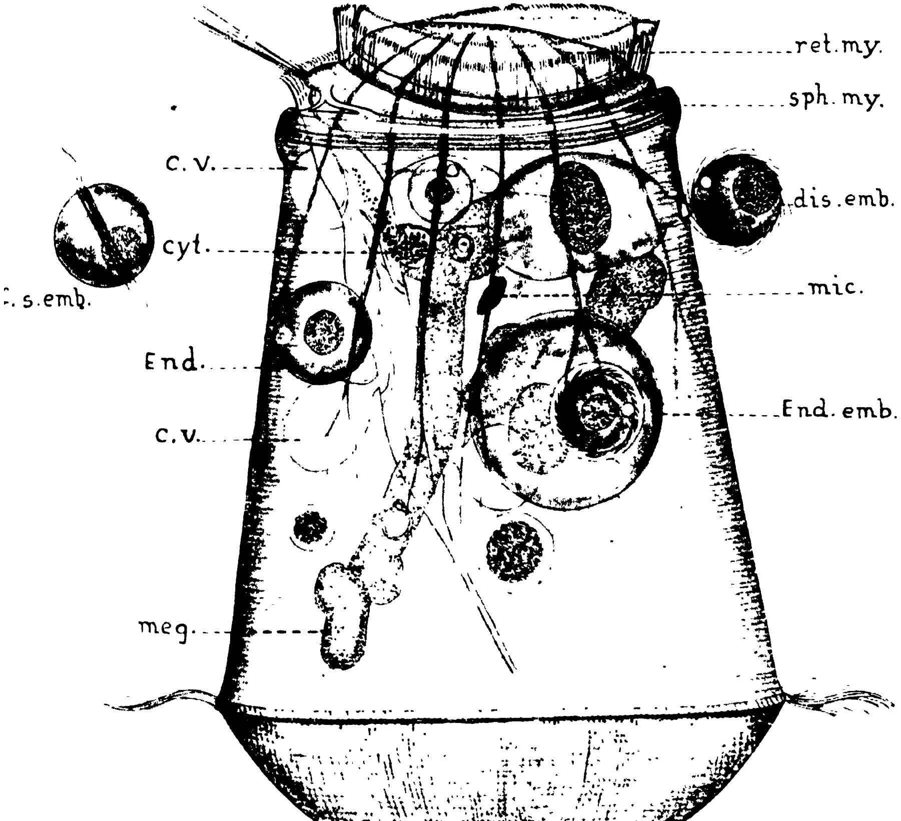
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PLATE 7

Fig. 1. Free-swimming embryo adherent to the surface of *Opisthonecta.* Section. Schaudinn's fluid. Iron-alum haematoxylin.

Fig. 2. Same.

Fig. 3. Embryo in early stage of penetration into *Opisthonecta.* Whole mount. Schaudinn's fluid. Iron-alum haematoxylin.

Fig. 4. Same; *c. v.*, contractile vacuole.

Fig. 5. Penetration of embryo half accomplished. Whole mount. Schaudinn's fluid. Iron-alum haematoxylin.

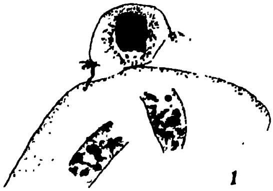
Fig. 6. Late stage of penetration. Section. Flemming's fluid. Iron-alum haematoxylin.

Fig. 7. Same. Whole mount. Schaudinn's fluid. Iron-alum haematoxylin.

Fig. 8. Young parasite without embryo. Section. Flemming's fluid. Iron-alum haematoxylin; *b. p.*, birth pore.

Fig. 9. Young parasite. Section. Schaudinn's fluid. Iron-alum haematoxylin; *meg.*, meganucleus; *mic.*, micronucleus.

Fig. 10. Young parasite. Section. Flemming's fluid. Iron-alum haematoxylin.



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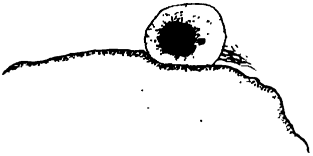

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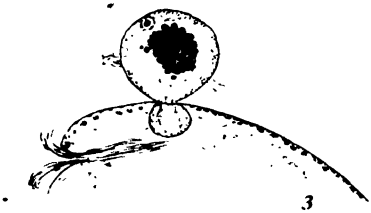

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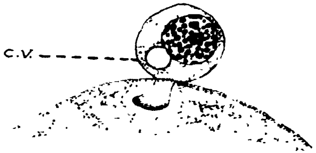

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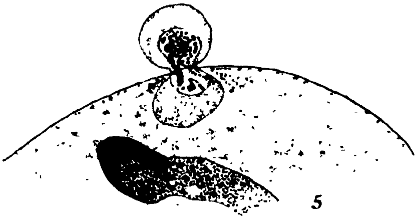

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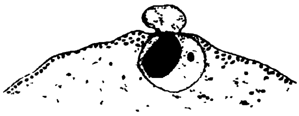

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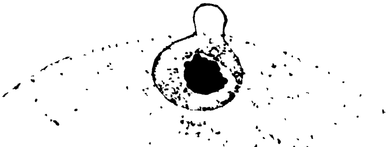

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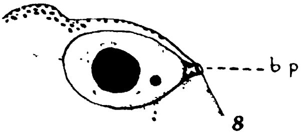

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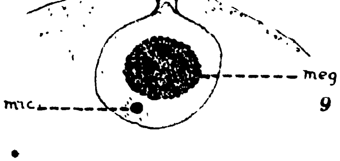

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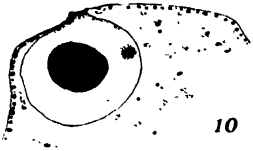

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PLATE 8

Fig. 11. Young parasites. Section. Flemming's fluid. Iron-alum hatematoxylin; *b. p. my.*, birth pore with radial myonemes; *b. p.*, birth pore.

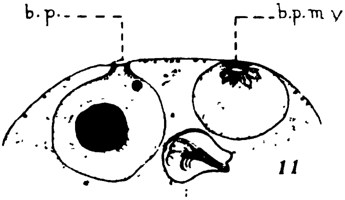
Fig. 12. Early stage of embryo formation. Whole mount. Schaudinn's fluid. Iron-alum haematoxylin.

Fig. 13. Embryo within brood chamber. Section. Flemming's fluid. Iron-alum haematoxylin; *b. p.*, birth pore. Note radial myonemes extending over wall of brood chamber.

Fig. 14. Early stage in emergence of embryo. Whole mount. Schaudinn's fluid. Iron-alum haematoxylin.

Fig. 15. Same.

Fig. 16. Discharge of embryo. Whole mount. Schaudinn's fluid. Borax carmine and indulin.



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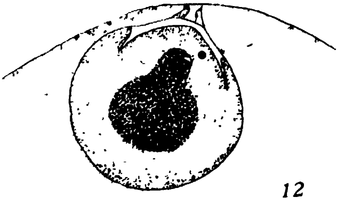

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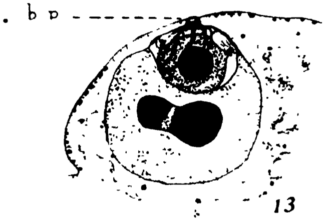

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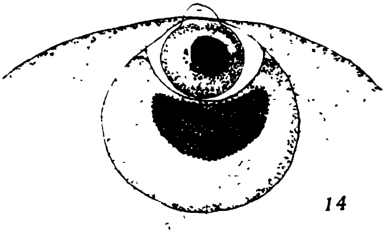

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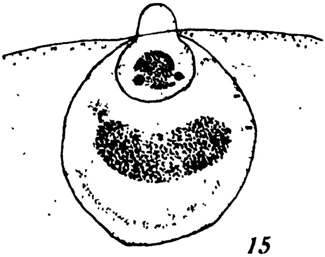

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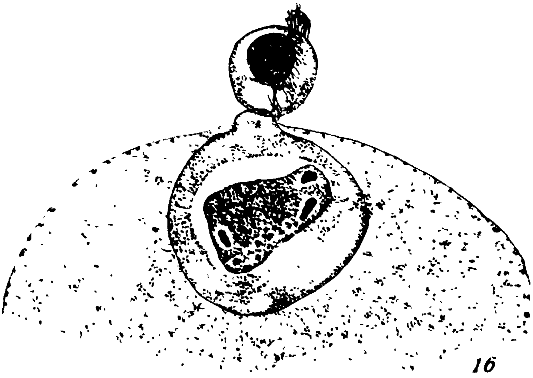

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A STUDY OF THE SPECIES OF EIMERIA OCCURRING IN SWINE

BY

DORA PRIAULX HENRY

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A STUDY OF THE SPECIES OF EIMERIA OCCURRING IN SWINE

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DORA PRIAULX HENRY

The question of the occurrence of more than one species of coccidium in domestic swine has been the subject of controversy for several years. In the most recent extensive article on coccidiosis in pigs, Biester and Murray (1929) reach the conclusion that there is but a single species. This conclusion is based entirely on a study of the size relationships of the oocysts. These authors plotted three hundred oocysts, from ten sources, as to the size of their circumscribed rectangles. They confirm the findings of Krediet (1921), who concluded that only one species is present in the pig, as the largest number of individuals fall in the middle group, and those at the two extremes are connected with this larger group by intermediate forms. The purpose of this paper is to show that size alone is not a reliable criterion of the number of species present.

The author wishes to acknowledge her indebtedness to Dr. Charles A. Kofoid for his interest in this work and to Mr. J. E. Gullberg for the microphotographs.

It does not seem necessary to review again the earlier work involving the controversy over nomenclature. An adequate review of the literature may be found in the paper by Biester and Murray (1929). The accepted designation of the commonly occurring coccidium of swine is *Eimeria debliecki* Douwes. From their descriptions *Eimeria suis* Nöller and *Eimeria brumpti* Cauchemez must be considered synonyms of *E. debliecki.*

In the original description of *Eimeria debliecki,* Douwes (1921) found that the oocysts varied in length from 17μ to 22μ, and in width from 14μ to 19μ. In a small number of cases he also found a large form with oocysts of 50μ by 35μ. Subsequent to this work, coccidia in swine have been reported from various parts of the world by

Nöller (1921), Krediet (1921), Cauchemez (1921), Nöller and Frenz (1922), Sheather (1923), Davis and Reich (1924), Munnik (1924), Frenz (1924), de Graaf (1925), Yakimoff (1926, 1927), and Biester and Murray (1929). In general, their descriptions correspond to that of Douwes for *E. debliecki.* The size ranges of the oocysts found by these authors are shown in table 1.

Of the above mentioned authors the majority reached the conclusion that there is but a single species. This was apparently based almost exclusively upon measurements of the oocyst, as the morphological description is in most cases extremely meager. By using the same criterion, namely, size, others of these workers have, paradoxically, suggested that the coccidia of swine should be separated into two or more species. In this latter group are Nöller and Frenz (1921), Frenz (1924), Sheather (1923), and de Graaf (1925).

Although, as mentioned above, several workers have recorded the occurrence of coccidia which they believe might represent more than one species, no attempt has been made to describe or name any species other than *E. debliecki.* Below we shall describe the three species of coccidia in addition to *E. debliecki,* which have been found in swine in California. The differentiation of these species from one another and from *E. debliecki* is based upon the morphological characters of the oocysts, and the sporulation time, in addition to size differences. Preliminary work upon experimental infection has indicated that further differences between the species will be found in their relation to the host.

The materials upon which this study is based were obtained in the majority of cases from the caecal contents of swine slaughtered at local packing houses. In a few instances faeces of infected animals held for observation were examined daily over an extended period. The incidence of infection was determined by direct faecal smears and not by the use of any of the flotation techniques for the concentration of cysts. The use of this cruder method seemed justified, since the object of this survey was to procure samples containing relatively large number of cysts. For this reason our percentage of infection in swine is not comparable to that found by other workers using a technique which would enable them to detect slight infections, undoubtedly overlooked with the direct smear method.

In our other work with the swine coccidia the sugar flotation technique of Sheather (1923) was employed to concentrate the cysts. Great difficulty was experienced in differentiating the oocysts of the several species in the sugar solutions as the characteristics of the cyst wall and the cytoplasm are obscured in this medium. The common use of sugar flotation techniques by workers studying swine coccidia may explain the failure to recognize these species heretofore.

Maximum sporulation in the shortest time was obtained when caecal contents or faeces containing the cysts were thoroughly mixed with a 2 per cent potassium bichromate solution and placed in Petri dishes or large moist chambers (7–11 inches in diameter) to a depth not exceeding one inch. In the case of liquid or semi-liquid specimens sufficient 5 per cent potassium bichromate solution was added to make a final dilution of the salt of approximately 2 per cent. By this method complete sporulation of *E. debliecki* occurred in from seven to nine days, as contrasted with the sporulation time of twenty-one days reported by Biester and Murray (1929). As this difference indicates, sporulation times found by various workers cannot be compared unless an identical procedure is followed. However, differences in the sporulation time of the several species are of considerable significance when the cysts are held under identical conditions. This comparison is most satisfactory when the various species are obtained in a single faecal sample and allowed to sporulate in the same container.

In order better to indicate the differences between *E. debliecki* and the three new species to be described, a brief account is given of the morphology of the oocysts and the incidence of the former species, as found by the writer.

Eimeria debliecki Douwes

Eimeria debliecki is undoubtedly the most common of the species of coccidia found in swine. It was first reported from California in 1924 by Davis and Reich, who found this species in five animals, material from which was obtained from the slaughter house. In the examination of materials from 162 animals from a similar source, 50, or 30 per cent, were found to be infected with *E. debliecki*. These animals all had their origin in California. An examination was made of caecal material from 45 animals raised in the Middle West but slaughtered in California, and *E. debliecki* was found in 37, or 82 per cent, of the cases.

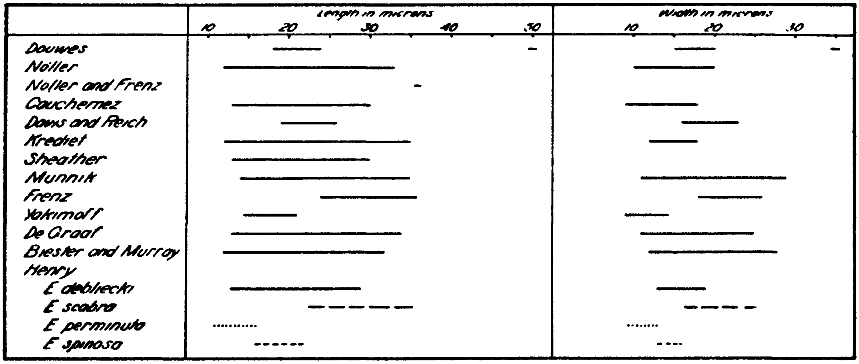
As shown in table 1, Davis and Reich found a size range of 19–26μ in length, and 16–23μ in width. The oocysts of this form which we encountered ranged in length from 12.8μ to 28.8μ, and in breadth from

12.8μ to 19.2μ. Fifty oocysts were measured from each of three swine, and the remainder of the 250 cysts upon which the above measurements were based were obtained from a number of other infections.

The ovoidal, only rarely spherical, cyst is surrounded by a double-contoured cyst wall which is 1μ or less in thickness and uniform throughout. In the smaller forms the cyst wall is colorless and transparent, whereas the larger forms frequently exhibit a slightly brownish wall. Similarly, the cytoplasm, which contains many small, evenly distributed granules, appears colorless in the smaller forms, while in the larger forms a greenish coloration is observed. In oocysts obtained directly from the caecum the cytoplasm is evenly distributed throughout the cyst. Within twenty-four hours after material containing the cysts has been placed in a 2 per cent solution of $K_2Cr_2O_7$ the cytoplasm rounds up into a rather regular spherical mass in the center of the cyst (pl. 10, fig. 15). At this time one or two, or in some cases a group of very small granules may usually be seen outside the cytoplasm (pl. 10, fig. 14). They are frequently found at one pole near the edge of the cyst.

TABLE 1

SIZE RANGES OF COCCIDIA OF SWINE AS FOUND BY VARIOUS WORKERS



When the technique described above is employed, sporulation in this species almost invariably begins on the fourth or fifth day. At this time sporoblasts are formed which first assume a square outline and then gradually round up, and when fully developed become ellipsoidal. In seven to nine days after the material is placed in potassium bichromate the sporulation process is completed with the formation of the sporozoites (pl. 10, figs. 14, 16).

Eimeria scabra sp. nov.

Oocysts of *Eimeria scabra* sp. nov. have been found in the caecal contents of 16 swine. Fourteen of these cases were from a group of 162 California animals. The remaining two were obtained from 45 swine which had been raised in Nebraska or Kansas and brought to California for slaughter. However, the possibility of infection with this species after arrival in California cannot be ruled out. *E. scabra* cysts were found in large numbers in the caecal material of nine cases of the sixteen mentioned above, and in lesser numbers in the remainder. In every instance a concurrent infection with *E. debliecki* was encountered. The numbers of cysts of the two species found in a single animal bore no relation to each other.

E. scabra may be readily differentiated from *E. debliecki* by the characteristic brown color of the cyst due to pigment contained in the wall, and by the definitely roughened wall (pl. 9, fig. 11; pl. 10, figs. 20, 22). This wall has an uneven outer surface (pl. 10, fig. 21) with unmistakably thickened areas more or less regularly arranged. This character together with the brown color gives the oocyst a very striking appearance. There is considerable variation in the roughness of the wall in oocysts from the same specimen. The wall of the oocyst of *E. scabra* is thicker than that of *E. debliecki;* it varies from 1.5μ to 2μ. The oocysts are ellipsoidal to very slightly ovoidal in shape, with a marked decrease in the thickness of the wall at one end, the smaller end in the case of the ovoidal cysts (pl. 9, fig. 12; pl. 10, figs. 13, 20). The size of the oocysts of *E. scabra* ranges from 22.4μ to 35.6μ in length, and from 16.0μ to 25.6μ in width.

In oocysts from fresh caecal contents the protoplasm fills the entire cyst (pl. 10, fig. 17), but as development proceeds it begins to contract, and at the end of two or three days forms a compact, roughly rectangular mass in the center of the cyst (pl. 9, fig. 11; pl. 10, fig. 19). At this time one or two polar granules may usually be found (pl. 9, figs. 12, 13; pl. 10, fig. 22). Although the protoplasm eventually (usually in three or four days) further contracts to form a sphere, the rectangular stage is more prolonged in this species than in *E. debliecki.*

In the faecal material from a six-weeks-old pig naturally infected with *E. scabra* and *E. debliecki,* oocysts of *E. scabra* were frequently found in which the cytoplasm had already rounded up. In daily examinations of the faecal material from this animal it was found that the number of cysts of both species varied considerably; in some specimens *E. debliecki* cysts were more numerous, while in others *E. scabra* predominated. During the period of eighteen days in which cysts were found, several specimens were examined which showed no cysts of either species. After eighteen days no cysts were found, although daily examinations were continued for some time and examinations were made at irregular intervals for several weeks. During the period in which *E. scabra* cysts were passed the average size of the cysts did not vary to any considerable extent.

When oocysts of *E. scabra* are allowed to sporulate by placing caecal material containing the cysts in a 2 per cent solution of potassium bichromate, sporoblasts are seen on the seventh or eighth day. In the same container cysts of *E. debliecki* begin to sporulate on the fifth or sixth day. The sporoblasts first appear square in shape (pl. 10, fig. 22), then round up, and finally become ellipsoidal, much as in *E. debliecki.* Fully sporulated cysts (pl. 9, figs. 12, 13; pl. 10, figs. 20, 21) can be found on the ninth to twelfth days, as contrasted with seven to nine days for *E. debliecki.* The sporocysts contain two sporozoites, residual material, and Stieda's bodies (pl. 10, fig. 20) at each end of the sporocyst. Usually, a plug filling the micropyle can also be seen. The sporocysts measure from 16μ to 19.2μ in length, and 6.4μ in width. A peculiarity of *E. scabra* which has never been noted by the author in any other species of *Eimeria* is its ability to sporulate without access to a large supply of oxygen. It has been noted in several instances that in smears made of fresh caecal material in which the coverslip was carefully sealed with paraffin, oocysts of *E. scabra* continued to develop and form mature sporocysts. This method of preparing slides has been used for preservation of non-sporulated oocysts of a variety of species of coccidia. Undeveloped oocysts of several species have been preserved in this manner for a period of four to five months.

A comparison of the measurements of oocysts given by various authors (see table 1) indicates that several of these workers may have worked with a mixed infection of *E. scabra* and *E. debliecki.* Nöller (1921) describes two types of oocysts. He states that the smaller öocysts, 12–15μ in length, have an unstained cyst wall, while the larger, 33μ in length, have a rough, yellowish cyst wall (*besitzen eine derbe* [rough?] *gelbliche Hülle*). The description of this latter form agrees with the characters of *E. scabra.* Nöller and Frenz (1922) discuss the possibility of there being two species of *Eimeria* in swine, but because they found forms intermediate in size between the small and large cysts and did not find any distinguishing morphological characteristics, they conclude that there is but a single species. The measurements given by Frenz (1924) coincide almost exactly with those of *E. scabra.* He found no difference in sporulation time nor in the appearance of the ripe oocyst other than that of size. Neither Nöller and Frenz (1922) nor Frenz (1924) mention the character of the cyst wall. De Graaf (1925) described a brown coloration of some of the larger oocysts which was not possessed by the smaller ones. He found no difference in sporulation time in the two forms.

Eimeria perminuta sp. nov.

From the caecal contents of one swine large numbers of small oocysts were obtained. These oocysts vary from 11.2μ to 16.0μ in length, and from 9.6μ to 12.8μ in width. The cysts are usually oval in shape, but frequently spherical (pl. 9, figs. 7–10). The wall is roughened in a manner similar to that of *E. scabra,* and is frequently yellow in color (pl. 9, fig. 8). Sporoblasts are not found until the ninth day and sporulation is not completed until the eleventh day (pl. 9, fig. 9). As no cysts of *E. debliecki* or *E. scabra* were found in this material, a direct comparison of their sporulation time with that of *E. perminuta* cannot be made. However, in three other specimens collected at the same time and treated in an identical manner, *E. debliecki* and *E. scabra* began to sporulate on the sixth and seventh days, respectively.

It will be noticed by a comparison of the measurements given in table 1 that there is a considerable gap between the smallest *E. scabra* (22.4μ) and the largest *E. perminuta* (16.0μ). This species is much more likely to be confused with *E. debliecki* than with *E. scabra,* even though it more nearly resembles the latter in morphological characteristics. Its size range coincides more closely with that of *E. debliecki,* and casual inspection of these minute cysts frequently fails to reveal the rough wall. It is possible that this species is responsible in some cases for the low range in size reported for *E. debliecki.*

Eimeria spinosa sp. nov.

This species was obtained from the caecal contents of an apparently normal California pig and from the caecum of a five-months-old pig which showed lesions similar to those found in enteritis of swine. This animal died immediately upon arrival from Minnesota. The material was procured through the kindness of Dr. Robert Jay, of the United States Bureau of Animal Industry.

The oocysts were fairly numerous in the material, which also contained a few cysts of *E. debliecki.* Because of the unique and exceedingly characteristic appearance of the oocysts (pl. 9, figs. 1–6), it seems very probable that the distribution of this species must be limited. If this were not the case, the species would probably have been previously described. The length of the cysts varies from 16.0μ to 22.4μ, and the width from 12.8μ to 16.0μ. The cysts are brown in color and the entire surface of the cyst wall is studded with spines approximately 1μ apart (pl. 9, figs. 1, 3, 5). The spines, which are wider at the base and taper to a point, originate at the inner surface of the cyst wall (pl. 9, fig. 6) and project 1μ beyond the outer surface.

The cytoplasm of the unsporulated cyst is extremely heavy in appearance and is made up of relatively large granules. After the cytoplasm has rounded up, granules similar to those found in other species of *Eimeria* may usually be found at the narrower pole (pl. 9, fig. 6). The rounded-up mass is usually found near the larger end of the cyst, rather than in the center, as in *E. debliecki* and *E. scabra.*

The material containing the cysts was placed in potassium bichromate and not examined until eight days had elapsed. At this time sporulated cysts of *E. debliecki* were found, but those of *E. spinosa* were still undeveloped. Sporulation (pl. 9, figs. 2, 3, 4, 6) began on the tenth day and was completed on the twelfth. The character of the sporocysts cannot be determined satisfactorily because of the color and the opacity of the cysts, caused by the spines. In mounts made in glycerine, Stieda's bodies and a large amount of residual material can be seen. The sporocysts measure from 9.1μ to 11.7μ in length, and from 5.2μ to 6.5μ in width.

SUMMARY

1. The incidence of *Eimeria debliecki* in swine slaughtered in California has been found to be 30 per cent for animals raised in California, and 82 per cent for animals raised in Nebraska and Kansas.

2. Three new species of coccidia occurring in swine, *Eimeria spinosa, Eimeria scabra,* and *Eimeria perminuta* have been described.

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EXPLANATION OF PLATES

Photomicrographs by J. E. Gullberg
Magnification × 1600

PLATE 9

Figs. 1–6. ***Eimeria spinosa*** **sp. nov.**

Fig. 1. Cyst showing spines in cyst wall.

Figs. 2–4. Sporulated cysts in which the spines are not so clearly seen. In figure 4 the oocyst is collapsed at one end.

Fig. 5. High focus of cyst wall showing the ends of the spines.

Fig. 6. Sporulated cyst. In upper left, polar granule seen. Spines can be seen between the two edges of the cyst wall.

Figs. 7–10. ***Eimeria perminuta*** **sp. nov.**

Fig. 7. Non-sporulated cyst with cytoplasm contracted.

Fig. 8. High focus of cyst wall showing the characteristic roughness.

Fig. 9. End view of sporulated cyst, with two sporocysts in focus. Polar granule also seen.

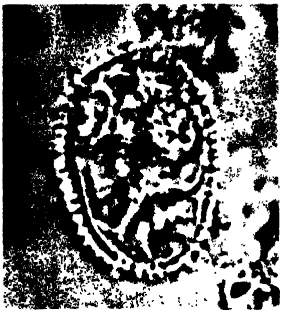
Fig. 10. Non-sporulated cyst, possible degenerate. Narrow form.

Figs. 11–13. ***Eimeria scabra*** **sp. nov.**

Fig. 11. Cyst with cytoplasm in center of cyst, but not fully contracted. Rectangular stage. The rough cyst wall can be seen.

Fig. 12. Sporulated cyst with two polar granules and thin cyst wall at narrower end.

Fig. 13. Mature cyst with two polar granules at one side of cyst.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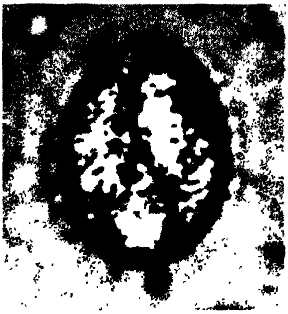
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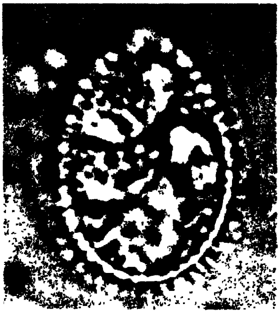
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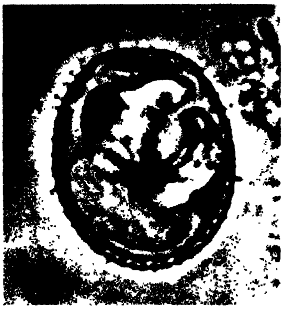
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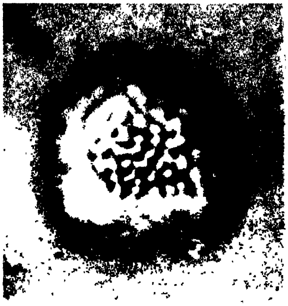
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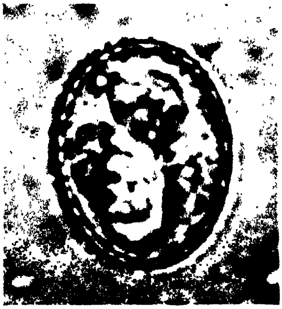
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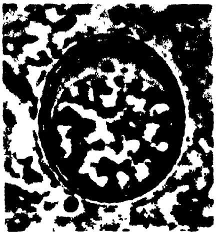
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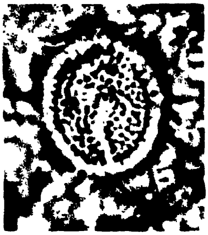
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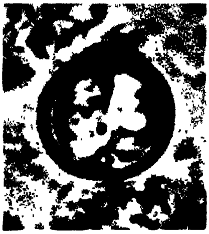
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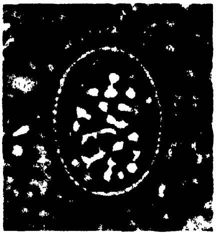
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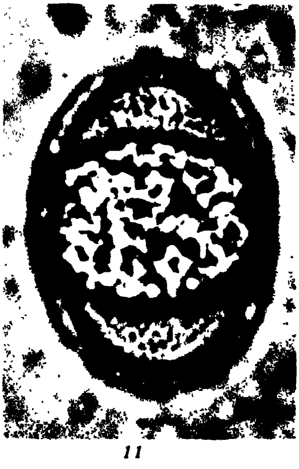
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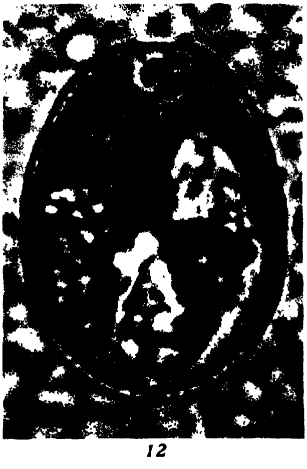
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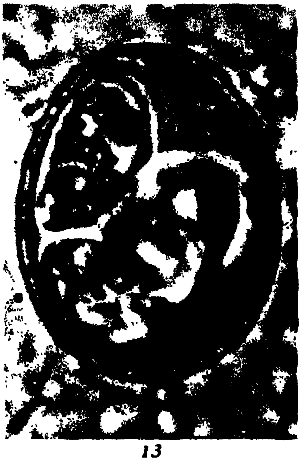
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PLATE 10

Figs. 14–16. *Eimeria debliecki* Douwes

Fig. 14. Sporulated cyst of smaller form with polar granule at one side of cyst.

Fig. 15. Cyst of larger form with rounded up cytoplasm. Clear cyst wall.

Fig. 16. Mature cyst.

Figs. 17–22. *Eimeria scabra* sp. nov.

Fig. 17. Immature form with cytoplasm completely filling cyst.

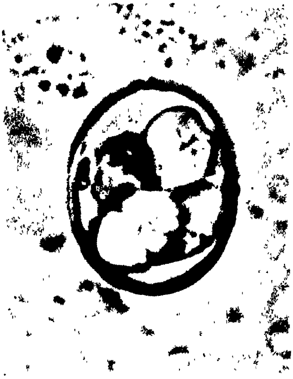
Fig. 18. Cyst with cytoplasm beginning to contract.

Fig. 19. Cyst with cytoplasm almost entirely contracted, nearly "ball" stage.

Fig. 20. Sporulated cyst. Stieda's bodies can be seen in the middle sporocyst.

Fig. 21. High focus of same cyst, showing the characteristic roughness of the cyst wall.

Fig. 22. Smaller form of *E. scabra.* Sporoblasts with square outline and polar granule immediately above center of cyst.



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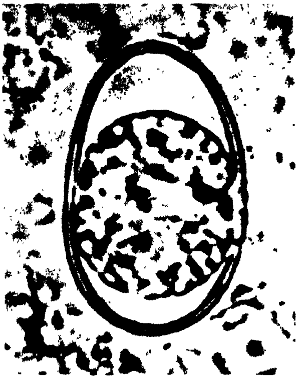

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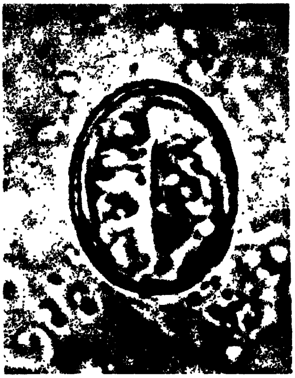

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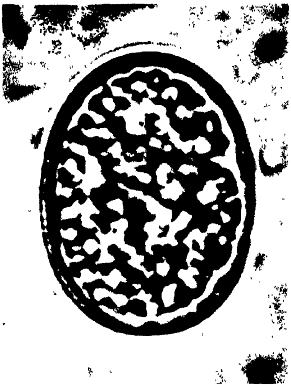

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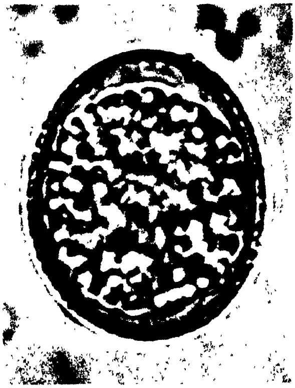

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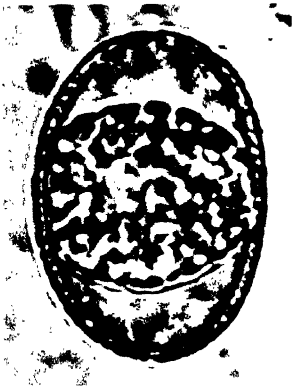

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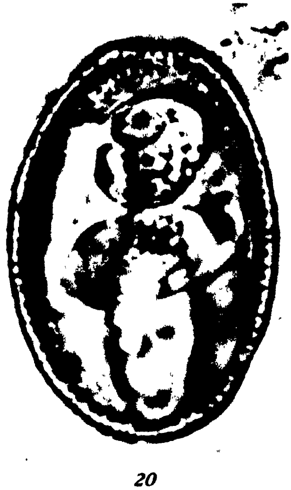

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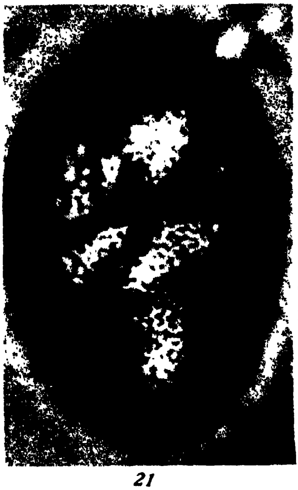

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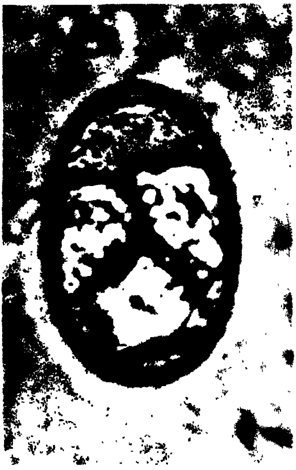

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THE WATER EXCHANGES OF LIVING CELLS

BY

JAMES L. LEITCH

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THE WATER EXCHANGES OF LIVING CELLS

I. THE NORMAL PERMEABILITY OF THE EGGS OF SOME MARINE INVERTEBRATES

BY

JAMES L. LEITCH

INTRODUCTION

The quantitative aspect of the process of swelling of marine eggs in diluted sea water was first studied by Lillie (1916). Several papers dealing with the water exchanges of invertebrate eggs have since been published by McCutcheon and Lucke and by Northrop. The rate of entrance of water into *Arbacia* eggs as affected by electrolytes, injury, salt concentration, pH, and temperature, has been studied by Blumenthal (1927); Lucke and McCutcheon (1927, 1929, 1930); McCutcheon and Lucke (1926*b*, 1928); and by Stuart (1928). Since all the data on the rate of swelling of marine eggs are derived from observations on one species only, the sea urchin *Arbacia punctulata,* it seemed desirable to study the eggs of other forms and to determine the physical and chemical nature of these eggs considered as osmotic systems. The present paper presents data on the rates of swelling of the eggs of several Pacific Coast invertebrates.

Grateful acknowledgment is made to Professor S. C. Brooks, of the University of California, at whose suggestion and under whose guidance this work has been done; to Dr. W. K. Fisher, Director of the Hopkins Marine Biological Station at Pacific Grove, for the use of the facilities of the Station; and to Professor Baldwin Lucke, of the University of Pennsylvania, for helpful criticisms and suggestions.

HISTORY

Lillie (1916, 1917, 1918*a* and *b*) studied both fertilized and unfertilized eggs of *Arbacia* with respect to their rate of volume increase with time when placed in diluted sea water. He expressed the rate of swelling by the constant, K, of the following equation:

$$K = \frac{1}{t} \ ln \ \frac{V_{eq} - V_o}{V_{eq} - V_t}, \tag{1}$$

where V_o, V_{eq}, and V_t are the initial volume, the equilibrium volume, and the volume at time t, respectively. The use of this equation was justified by the approximate constancy of K during the process of swelling. There was, however, a perceptible tendency for K to decrease as swelling progressed.

Northrop (1927*c*) found that the intake of water into *Arbacia* eggs and other tissues obeyed the same equation as that expressing the entrance of water into electrolyte-free gelatin (Northrop, 1927*a* and *b*). This equation is:

$$C = \frac{hV_e}{SP_o t}\left(V_o - V_t + 2.3 \ V_e \log \frac{V_e - V_o}{V_e - V_t}\right), \tag{2}$$

where V_o, V_t, and V_e are the initial volume, the volume at time t, and the equilibrium volume, respectively; P_o is the internal osmotic pressure; S is the surface area; and h is the thickness of the layer of gelatin. The constant C expresses the number of cubic centimeters of water passing through one square centimeter of surface, one centimeter thick, in one hour under a pressure of one millimeter of mercury. When this equation is applied to the swelling of eggs, h represents the thickness of the cell membrane. Northrop considers this to be constant and therefore expresses the permeability by the constant term, C/h.

Lucke and McCutcheon (1926) and McCutcheon and Lucke (1926*a*, 1927) pointed out that Lillie's equation is actually one form of the unimolecular reaction isotherm and can be applied to both the swelling and the shrinking of *Arbacia* eggs. More recently, however, McCutcheon and Lucke (1928) and Lucke and McCutcheon (1930) have used an equation expressing the permeability in absolute units as follows:

$$\text{Permeability} = dV/dt \Big/ S(P - P_{ex}), \tag{3}$$

where dV/dt is the rate of volume change with time; S is the surface area; P is the osmotic pressure at any time; and P_{ex} is the osmotic pressure of the external solution. The permeability is, therefore, the number of cubic microns of water entering one square micron of egg surface in one minute under an osmotic pressure of one atmosphere. In this paper we follow the usage established by McCutcheon and Lucke, taking the rate of volume change at the third minute as the most reliable measure of the permeability. Experience shows that values so derived are valid within 1 per cent. Further discussion of the reasons for this usage is postponed to a future paper.

EXPERIMENTAL PROCEDURE

Material.—The starfish, *Pisaster ochraceous*, was collected from the tide-pools of Moss Beach, San Mateo County, California, and used at Berkeley. The ovaries were removed, placed in sea water, and gently shaken. As with all material, the eggs were washed free of ovarian tissues and other débris and then allowed to stand in normal sea water for half an hour, or preferably for one hour, before being used.

The remainder of the material was collected in and around Monterey Bay, *Strongylocentrotus* during December and the other forms during June and July, and used at the Hopkins Marine Station at Pacific Grove. The preparation of the eggs of the starfish, *Patiria miniata*, and of the sea urchins, *Strongylocentrotus franciscanus* and *S. purpuratus*, was the same as that described for *Pisaster*.

Urechis caupo.—This is a marine worm of uncertain systematic position now appended to the Phylum Annelida. The eggs, which accumulate in modified excretory organs, were removed as needed by means of a fine pipette introduced through the pores which constitute their normal place of exit.

Dendraster excentricus.—The eggs of this sand-dollar were obtained by cutting open the whole animal and removing the ovaries to a dish of sea water. Unlike the other eggs, these are surrounded by a very noticeable jelly. In order to find out whether this jelly had any effect on the permeability to water, the following modification of a well-known procedure was adopted. After the eggs had been freed from sea water, they were placed in a 5/8M NaCl solution for two minutes. They were next shaken gently for five or ten seconds and then returned to sea water. If the shaking were neither too long nor too violent, some of the eggs were found without the surrounding jelly while others retained it. Both groups of eggs were capable of normal fertilization and development, showing that they had not been materially injured by this treatment. This permitted the determination of the rate of entrance of water into eggs both with and without their gelatinous envelopes, both groups having been exposed to the same conditions.

It is very necessary in all experiments of this type that the eggs be allowed to adjust themselves to the normal sea water. This is especially true for *Patiria* and *Urechis* since their eggs, when first removed, are angular in shape. An hour's immersion in sea water is usually long enough to cause them to assume the spherical shape.

Determination of the rate of swelling.—The studies were made in different dilutions of sea water referred to as 40 per cent, 50 per cent, and 60 per cent. These dilutions were made by adding sufficient distilled water to normal sea water to reduce the salt concentration of the latter, to 40 per cent, 50 per cent, or 60 per cent of its original value. To ten cubic centimeters of diluted sea water, contained in a Syracuse watch glass, approximately one-tenth of a cubic centimeter of egg suspension was added with thorough mixing. A single egg was selected and its diameter measured with an ocular micrometer at intervals of one minute. A series of readings up to and including the tenth minute supplied adequate data for the calculations.

It is necessary that the eggs measured be spherical; therefore the measurements of any egg which failed to remain spherical throughout the entire ten minutes were discarded. This must be done, for otherwise the volumes calculated from the measurements would bear an indefinite relation to the true volumes and would therefore be meaningless. It is also advisable that the eggs do not vary greatly in their initial volume. A complete experiment for the eggs of any one species in a given concentration consisted of twenty-five experiments of this type.

The temperature was measured and kept constant within two degrees. In the case of *Arbacia,* the change in permeability resulting from this amount of variation is about the same as the probable error due to the individual variations among eggs. There was nothing in our experiments to indicate any greater effect of temperature on the species we studied.

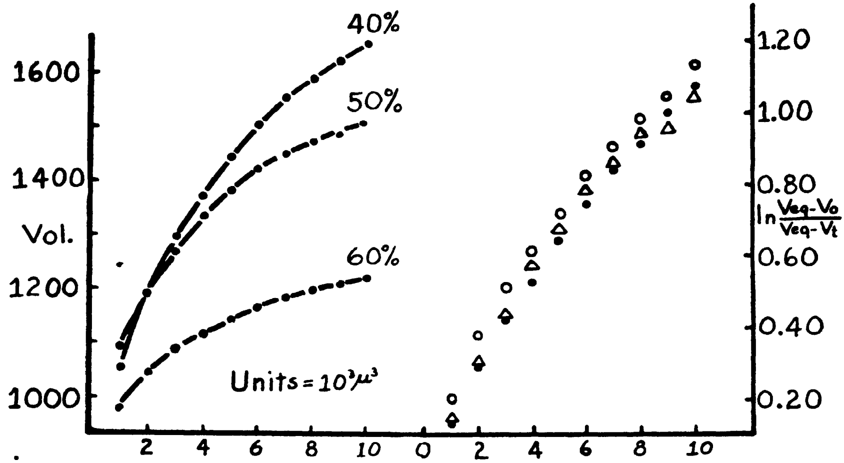


Fig. 1. *Dendraster excentricus.* *a,* The ordinates represent the volumes V_t at time *t,* plotted against the time (abscissas). *b,* The relationship between $ln\ \frac{V_{eq}-V_o}{V_{eq}-V_t}$ (ordinates) and time (abscissas). The symbols represent the concentration of sea water as follows: ● = 40 per cent; △ = 50 per cent; O = 60 per cent.

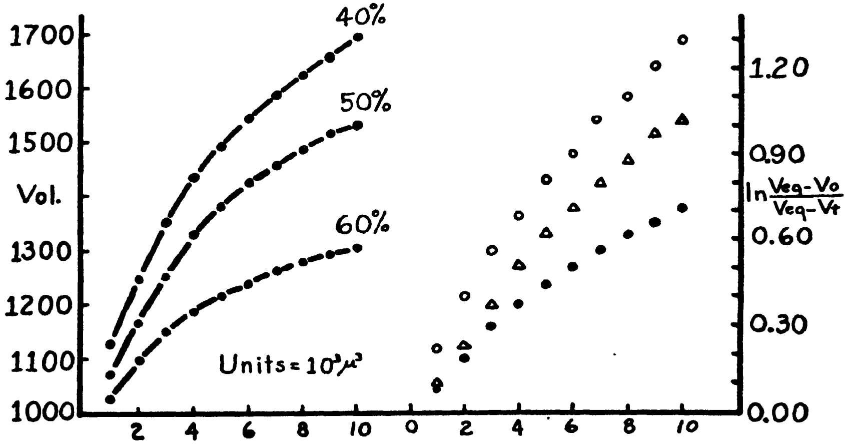


Fig. 2. *Dendraster excentricus.* (Jelly removed) *a,* The ordinates represent the volumes V_t at time *t,* plotted against the time (abscissas). *b,* The relationship between $ln\ \frac{V_{eq}-V_o}{V_{eq}-V_t}$ (ordinates) and time (abscissas). The symbols represent the concentration of sea water as follows: ● = 40 per cent; △ = 50 per cent; O = 60 per cent.

METHODS OF CALCULATING PERMEABILITY

In calculating the permeability, the following three assumptions are made:

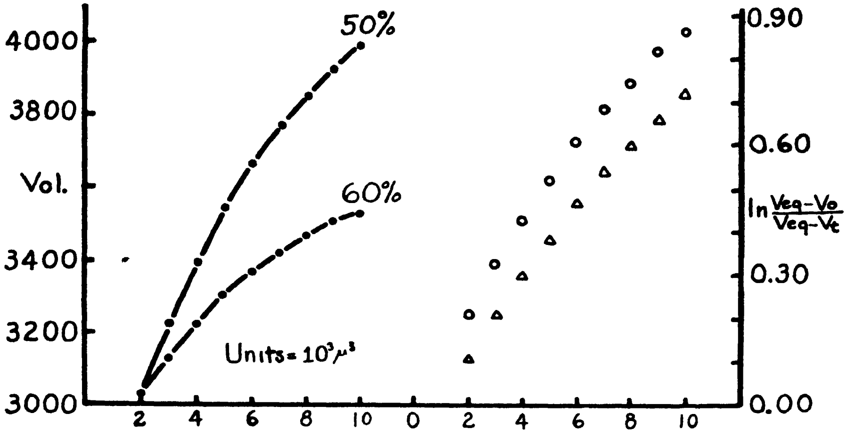
(1) During the swelling, the exchange of water is considered to be the only process taking place. That there might be an ex- or endosmosis of salts or other solutes is readily admitted, but from what we know of the permeability of living cells in general with respect to substances other than water, it seems improbable that processes of this kind have any significant effect during experiments of such short duration as ours.

(2) Of the total volume of the egg, 85 per cent is assumed to be osmotically active. This is the value deduced by McCutcheon and Lucke (personal communication) from observations on the swelling of *Arbacia* eggs; it is used here, since no figures are yet available for the forms under consideration.

(3) The product of the osmotic pressure within the egg and its osmotically active volume is assumed to be constant, ($\pi V = RT$).

When Lillie's equation is used, the mean of the observed diameters of the eggs at any given minute is calculated and the corresponding volume is computed. When these volumes are plotted against time (see figs. 1*a*–7*a*) a smooth curve results, from which the value for the initial volume, V_o, is found by extrapolation. The value for the equilibrium volume is found by multiplying the osmotically active volume by the reciprocal of the concentration and then adding the osmotically inactive volume to the product, the concentration of normal sea water being taken as unity. The values for the constant as given in table 1 represent the average of the constants up to and including the tenth minute.

When equation (3) is used, the value for dV/dt is taken as the slope of the V–t curve at the third minute. The osmotic pressure of the external solution, P_{ex}, is calculated from that of normal sea water at Pacific Grove (23 atmospheres according to Bigelow and Leslie, 1930). The osmotic pressure within the egg at any time can readily be found from the observed volumes on the assumption already mentioned that for these eggs the relationship $\pi V = RT$ is valid. In table 2 the permeabilities of the eggs of the species studied are given in terms of the constants calculated in this way.



·Fig. 3. *Patiria miniata.* *a,* The ordinates represent the volumes V_t at time t, plotted against the time (abscissas). *b,* The relationship between $ln\ \frac{V_{eq}-V_o}{V_{eq}-V_t}$ (ordinates) and time (abscissas). The symbols represent the concentration of sea water as follows: ● = 40 per cent; △ = 50 per cent; O = 60 per cent.

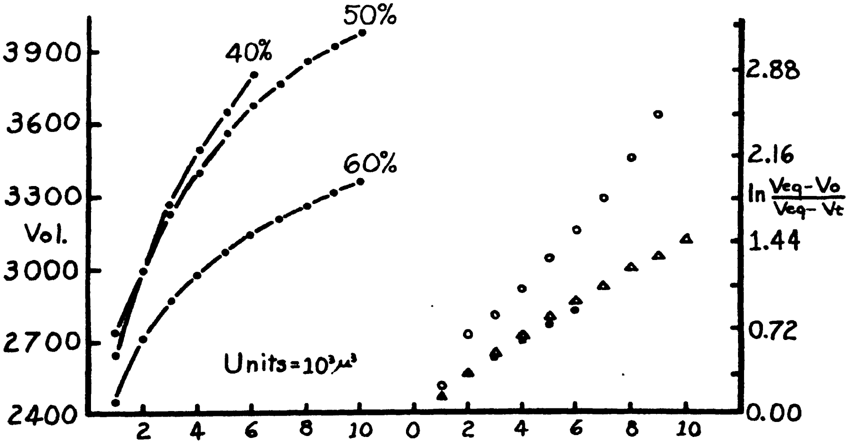


Fig. 4. *Pisaster ochraceous.* *a,* The ordinates represent the volumes V_t at time t, plotted against the time (abscissas). *b,* The relationship between $ln\ \frac{V_{eq}-V_o}{V_{eq}-V_t}$ (ordinates) and time (abscissas). The symbols represent the concentration of sea water as follows: ● = 40 per cent; △ = 50 per cent; O = 60 per cent.

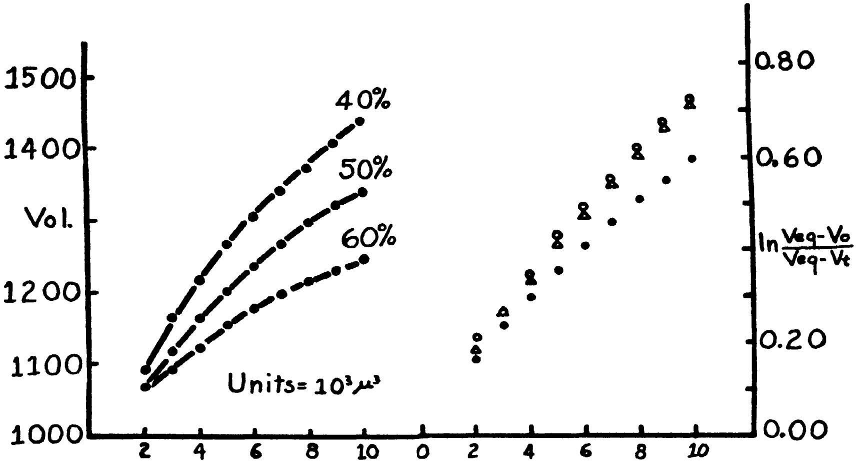


Fig. 5. *Strongylocentrotus franciscanus.* *a,* The ordinates represent the volumes V_t at time *t,* plotted against the time (abscissas). *b,* The relationship between *ln* $\frac{V_{eq} - V_o}{V_{eq} - V_t}$ (ordinates) and time (abscissas). The symbols represent the concentration of sea water as follows: ● = 40 per cent; △ = 50 per cent; ○ = 60 per cent.

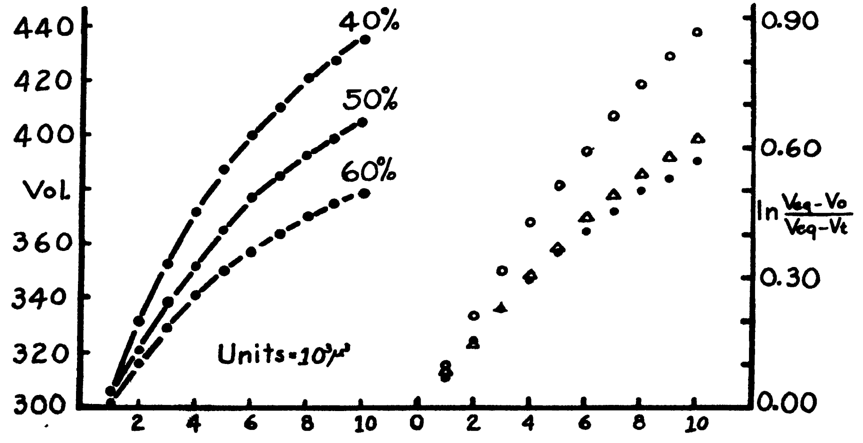
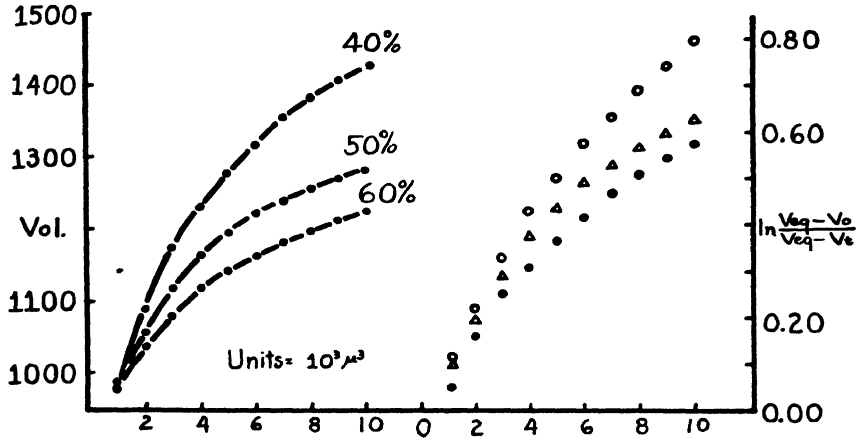


Fig. 6. ***Strongylocentrotus purpuratus.*** ***a,*** **The ordinates represent the volumes V_t at time *t,* plotted against the time (abscissas).** ***b,*** **The relationship between *ln* $\frac{V_{eq} - V_o}{V_{eq} - V_t}$ (ordinates) and time (abscissas). The symbols represent the concentration of sea water as follows: ● = 40 per cent; △ = 50 per cent; ○ = 60 per cent.**



. Fig. 7. *Urechis caupo.* *a*, The ordinates represent the volumes V_t at time *t*, plotted against the time (abscissas). *b*, The relationship between $ln \frac{V_{eq}-V_o}{V_{eq}-V_t}$ (ordinates) and time (abscissas). The symbols represent the concentration of sea water as follows: ● = 40 per cent; Δ = 50 per cent; O = 60 per cent.

TABLE 1

The Average Velocity Constant for the Rate of Swelling of the Eggs of Six Pacific Coast Invertebrates as Calculated by Lillie's Equation. Published Values for *Arbacia* are Given for Purposes of Comparison. Each Value is Based upon the Measurement of 25 Eggs.

	K			Temperature
	In 40 per cent sea water	In 50 per cent sea water	In 60 per cent sea water	
Dendraster excentricus:				
With jelly	0.128	0.131	0.148	20–22° C
With jelly removed	0.083	0.114	0.159	20–22
Patiria miniata	....	0 073	0.099	20–22
Pisaster ochraceous	0.159	0.165	0.273	20–22
Strongylocentrotus franciscanus	0.069	0.081	0.084	17–19
S. purpuratus	0.068	0.072	0.100	17–19
Urechis caupo	0.067	0.082	0.097	20–22
Arbacia (Lillie)	0.104	.....	....	....
Arbacia (McCutcheon and Lucke)	0.012	....	0.024	15
	0.021	....	....	17.6
	0.024	....	....	20.5

TABLE 2

The Permeability to Water of the Eggs of Six Pacific Coast Invertebrates (Column 6), Calculated in Accordance with McCutcheon and Lucke, from the Rates of Volume Increase, the Surface Areas, and the Osmotic Gradients at the Third Minute as Given in Columns 3, 4, and 5. The Permeability of *Arbacia* Eggs is Given for Purposes of Comparison. Each Value Represents Measurements of 25 Eggs.

1	2 Conc.	3 dV/dt	4 S	5 P—P_{ex}	6 Perm.
Dendraster excentricus	40%	94.5	60.34	5.90	0.266
	50%	75.4	59.49	5.43	0.234
	60%	38.7	53.66	3.52	0.204
Dendraster excentricus (jelly removed)	40%	92.2	62.00	7.55	0.197
	50%	81.7	58.95	6.06	0.228
	60%	43.6	55.66	4.07	0.195
Patiria miniata	50%	188.3	105.50	7.88	0.226
	60%	98.0	94.62	5.60	0.185
Pisaster ochraceous	40%	238.7	111.90	5.38	0.397
	50%	214.3	110.80	4.98	0.389
	60%	133.9	102.50	2.92	0.442
Strongylocentrotus franciscanus	40%	62.3	53.51	8.25	0.141
	50%	50.0	52.11	7.20	0.133
	60%	34.8	51.30	6.10	0.110
S. purpuratus	40%	19.6	23.93	8.42	0.097
	50%	16.6	24.40	7.54	0.090
	60%	11.9	23.04	5.68	0.091
Urechis caupo	40%	67.3	56.29	8.13	0.147
	50%	51.9	54.52	6.89	0.138
	60%	38.6	53.38	5.58	0.130
Arbacia (M. & L.)	....	....	....	....	0.05–0.06

DISCUSSION

If K in Lillie's equation were truly constant, then the curve relating the logarithms of the volumes to the time should be a straight line. It becomes quite evident from figures 1*b*–7*b* that for the eggs studied there is not a rectilinear relationship of this type. Such a relationship is, however, rather closely approached by some of the species, especially during the second half of the swelling process. The average velocity constants, as shown above, fall into two groups: one, comprising *Dendraster* and *Pisaster,* with velocity constants in 40 per cent sea water greater than 0.08; the others with values below that figure. From our present knowledge of this subject, it is not worth while offering any explanation of this condition. However, in the light of the variations in the constant with temperature as found by McCutcheon and Lucke (1926*a*), it does not seem likely that temperature can be responsible for so great a difference. In these experiments the variation of the permeability constant with concentration is approximately one-third of that found for *Arbacia.*

It is much better to compare the volumes of water entering cells in unit time under standard conditions than it is to use the velocity constants of equation (1) for the rates of entrance of water. In the first case, the comparison is between the number of cubic microns of water entering one square micron of egg surface in one minute under a driving pressure of one atmosphere. In contrast to this, Lillie's equation considers only the volumes and does not allow for changes in surface area. The permeabilities of the eggs, calculated according to the equation of McCutcheon and Lucke, are summarized in table 2. These values may have to be modified later, since they are based on the assumption that 85 per cent of the egg volume is osmotically active. The decrease of permeability, as thus calculated, with decrease in concentration, is quite probably due to the fact that the osmotically active volume differs from the assumed value of 85 per cent. Since incorrect assumptions as to the magnitude of the osmotically active volume affect the absolute values of the permeability, the apparent differences between species, as indicated in table 2, might be due, in part, to differences in this factor. Determinations of the osmotically active volume of the eggs of all species are now being made. Upon completion of this work it will be possible to introduce appropriate corrections of the calculated permeability.

It is obvious that the eggs of the Pacific Coast forms have much greater permeability than those of *Arbacia.* The permeability of the eggs of *S. purpuratus* comes nearest that of *Arbacia,* being between 0.090 and 0.100 for the former, as compared with 0.050 and 0.060 for the latter. There appears to be very little difference between *S. franciscanus* and *Urechis,* the eggs of both being rather more than twice as permeable as those of *Arbacia.* The permeabilities of the eggs of *Dendraster* and *Patiria* are in the neighborhood of 0.200–0.250, the presence of the enveloping jelly having no appreciable effect on the permeability of the eggs of the former. This latter statement accords with the idea that the function of the jelly is merely to protect against mechanical injury and that it is not involved in regulating the intake or outflow of water. The case of *Pisaster* is somewhat unique in that its permeability is from six to seven times that of *Arbacia.*

SUMMARY

1. The equation $K = \frac{1}{t}\ ln\ \frac{V_{eq} - V_0}{V_{eq} - V_t}$ has been applied to the rate of swelling of the eggs of several marine invertebrates, but does not fit the data so well as in the case of *Arbacia* eggs.

2. The average velocity constant K is from three to eight times that calculated for *Arbacia* by McCutcheon and Lucke. It increases as the concentration of the sea water increases, but much more slowly than in the case of *Arbacia.*

3. The permeability has been calculated, as suggested by McCutcheon and Lucke, in terms of cubic microns per square micron, per minute, per atmosphere, and found to be from two to eight times as high for these forms as for *Arbacia.*

4. The jelly enveloping the eggs of *Dendraster* has no appreciable effect on their permeability to water.

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A HOLOTRICHOUS CILIATE FROM THE INTESTINE OF ACMAEA PERSONA ESCHSCHOLTZ

BY

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THE MORPHOLOGY OF EUPOTERION PERNIX GEN. NOV., SP. NOV., A HOLOTRICHOUS CILIATE FROM THE INTESTINE OF ACMAEA PERSONA ESCHSCHOLTZ

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INTRODUCTION

While examining the intestinal contents of *Acmaea persona,* the mask limpet, numerous small ciliates were noted swimming vigorously through the slimy mass. Examination proved them to be an interesting holotrich allied to *Conchophthirius* Stein and *Cryptochilum* Maupas. We have described this ciliate under the name *Eupoterion pernix* gen. nov., sp. nov.

The original material for this study was collected in Golden Gate Park, San Francisco, and further material was obtained at Stinson's Beach, California. Thanks are due to Miss Elizabeth Stevenson for collecting and to Mr. E. W. Dennis for aid in preparation of the original material.

This study was made in the Zoological Laboratory of the University of California at Berkeley. We wish to acknowledge Dr. C. A. Kofoid's helpful suggestions and criticism of the manuscript.

METHODS

The shell of *Acmaea persona* was removed carefully with a keen scalpel and the intestine slit open. The contents were transferred to slides by means of a pipette. Smears of the intestinal contents were fixed in hot Schaudinn's fluid (60° C) and stained with Heidenhain's iron haematoxylin. To other smears a coverglass was applied and a study made of living material.

OBSERVATIONS ON LIVING MATERIAL

The oral surface is considered as ventral in accordance with Hentschel's (1924) orientation of *Cryptochilum.* All designations such as dorsal, ventral, etc., used in this paper are in accordance with this scheme.

Eupoterion pernix is nearly oval in lateral view with the small end anterior. The anterior half of the dorsal surface is nearly plane but the posterior half becomes convex and the curvature increases rapidly toward the posterior end of the body (fig. A, *2*). Anteriorly, the ventral surface is slightly convex (fig. A, *2*) and becomes more and more strongly convex as it proceeds posteriorly. As seen in lateral view and optical section the posterior third of the body appears nearly semicircular. In dorsal view, the right surface is slightly convex anteriorly, with the curvature increasing gradually to the posterior end (fig. A, *1*). The left surface, however, is slightly concave from near the anterior end to the posterior third of the body where the curvature reverses and becomes sharply convex (fig. A, *1*). Thus *Eupoterion* is slightly flattened laterally, the transverse diameter being about 0.7 dorso-ventral diameters.

The *ciliary lines* run antero-posteriorly without evidence of any tendency to spiral. The prominent oral cilia are about twice the length of the peripheral cilia and are best seen in life. Though the peripheral cilia are not particularly long (5–8μ) the organism swims with great speed and power.

Eupoterion usually proceeds in a straight line, rapidly spinning in a clockwise direction on its longitudinal axis. Occasionally it will proceed slowly for a short distance, revolving counterclockwise, but soon reverts to the former method. At times it swims rapidly about in small circles and then darts off so quickly that it is nearly impossible to keep the organism in the microscopic field at 250 diameters magnification. When the intestinal contents are thick, *Eupoterion* often leaves behind it a trail of rearranged particles, which, for a minute or two, remain to mark its path.

The *cytostome* lies in the postero-ventral margin of the body. Its position is marked in life by the long, powerful peristomial cilia which curve around it.

The *ectoplasm* is clear and is thickest anteriorly (fig. B, *2, ectopl.*). The *endoplasm* (fig. A, *2,* fig. B, *2, endopl.*) is clearly granular and only slightly vacuolated anteriorly. The micronucleus, lying in the endoplasm anterior to the macronucleus, can be identified as a small, greenish granule (fig. A, *2*, *micron.*). The macronucleus occupies the greater portion of the anterior half of the body. Its outline (fig. A, *2*, *macron.*) is made visible by its displacement of the denser endoplasm which surrounds it. In the posterior half of the body, the endoplasm is highly vacuolated, many of the vacuoles containing particles of food of varying size. The contractile vacuole (fig. A, *2*, *con. vac.*) lies in the extreme posterior region of the body just dorsal to the cytopharynx. It is flask shaped, or ovoid, with the small end near to, or in contact with, the pellicle. It can be seen to advantage only in life, for after fixation it cannot be distinguished from the endoplasmic vacuoles. It contracts infrequently; the fastest time recorded was five minutes in an animal observed in material diluted with sea water.

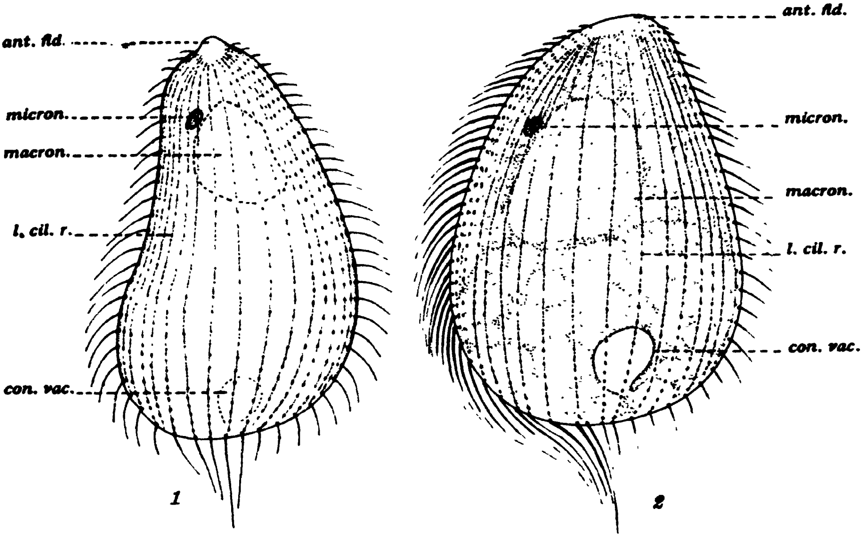

Fig. A. *Eupoterion pernix* gen. nov., sp. nov. Drawn from living material; × 1000. *1*, dorsal view; *2*, lateral view. *ant. fld.*, anterior field; *con. vac.*, contractile vacuole; *l. cil. r.*, longitudinal ciliary row; *macron.*, macronucleus; *micron.*, micronucleus.

Eupoterion pernix dies very soon after removal from its host. Most of the organisms were dead within fifteen minutes from the time they were mounted on slides.

The first observations on living material were made from our original collection. Further specimens of *Acmaea* from Stinson's

Beach, California, were collected to determine the extent of the infection and to extend the observations upon living material. All the limpets collected showed heavy infections.

SIZE

The *ciliate* is relatively small. Ten specimens selected at random from the fixed material measured 51μ (38–58μ) in length and 33μ (25–40μ) on the dorso-ventral axis. There is a slight lateral flattening, the transverse axis being 0.7 times the length of the dorso-ventral axis, far less than Hentschel (1924) reports for *Cryptochilum boreale*. Only a few satisfactory measurements of this lateral flattening could be made on the living ciliates because of their incessant activity. In fixed material this compression is either reduced or accentuated, depending on the position of the individual in the smear.

CYTOSTOME AND CYTOPHARYNX

The narrow, triangular oral groove (fig. B, *1, or. grv.*) originates on the mid-ventral surface, gradually deepens as it proceeds posteriorly and finally merges with the cytostome (fig. B, *2, cyto.*) which opens on the postero-ventral surface of the body. A small conical cytopharynx, lined with fibrils, extends inward from the mouth and continues anteriorly toward the macronucleus as a compact bundle of fibrils, the pharyngeal strand (fig. B, *2, ph. str.*). One of these fibrils is a continuation of the transverse fibril (fig. B, *2, trans. fibr.*) from the neuromotorium, a more direct connection to the neuromotorium than in the case of the pharyngeal fibril of *Boveria teredinidi* described by Pickard (1927). The other fibrils (fig. B, *2, ph. fibr.*) of the pharyngeal strand arise from the posterior loop of the main peristomial fibril, apparently from the basal granules. The pharyngeal strand has been reported in diverse groups of ciliates, and in all groups it apparently functions as a reenforcement of the pharyngeal wall.

CYTOPLASM

A thin, but highly resistant *pellicle* covers the cytosome. It appears to be perfectly plain with none of the complex sculpturing found in such forms as *Paramoecium*. A careful study of individuals in anterior view and in optical section in both living and fixed material shows the pellicle as a smooth surface, and the cilia as not arranged on grooves or ridges.

The *ectoplasm* (fig. B, *2*, *ectopl.*) is a clear granular layer directly beneath the pellicle, 10–15μ thick at the anterior end of the body, but tapering to 3μ or 4μ in thickness at the posterior end. The basal granules of the cilia and the complex fibrils of the neuromotor system lie within the ectoplasm.

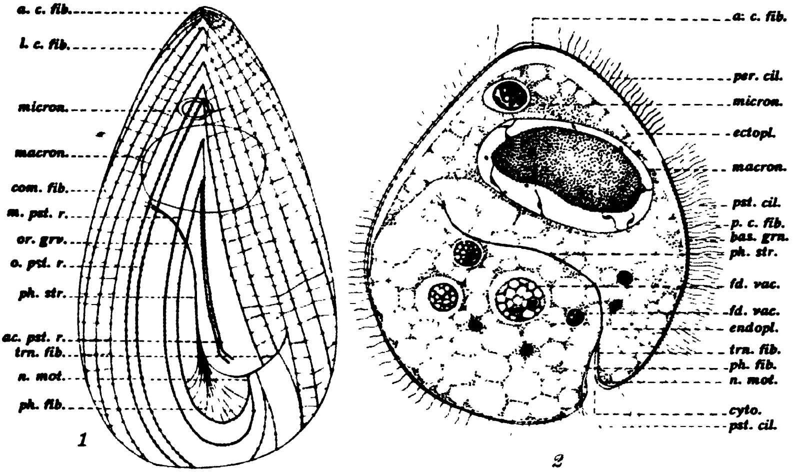

Fig. B. *Eupoterion pernix* gen. nov., sp. nov. Drawn with aid of camera lucida; × 980. *1*, neuromotor system from the ventral side; *2*, optical section. *a. c. fib.*, anterior connective fibril; *ac. pst. r.*, accessory peristomial ciliary row; *bas. grn.*, basal granule; *cyto.*, cytostome; *ectopl.*, ectoplasm; *endopl.*, endoplasm; *fd. vac.*, food vacuole; *l. c. fib.*, longitudinal connective fibril; *macron.*, macronucleus; *micron.*, micronucleus; *m. pst. r.*, main peristomial row; *n. mot.*, neuromotorium; *o. pst. r.*, outer peristomal ciliary row; *or. grv.*, oral groove; *p. c. fib.*, peristomial connective fibril; *per. cil.*, peripheral cilia; *ph. fib.*, pharyngeal fibril; *ph. str.*, pharyngeal strand; *pst. cil.*, peristomial cilia; *trn. fib.*, transverse fibril.

The strikingly vacuolated *endoplasm* (fig. D, *2*, *endopl.*) comprises the great bulk of the cytosome. That portion of the endoplasm which extends from the anterior end of the body slightly beyond the posterior margin of the macronucleus is relatively homogeneous and contains few vacuoles. The remainder of the endoplasm is highly vacuolated and contains the numerous food particles that have been engulfed.

NUCLEI

Eupoterion pernix possesses one macronucleus and one micronucleus. The micronucleus (fig. B, *2*, *micron.*) lies free in the dense endoplasm in the anterior sixth of the body during the interphase ("resting stage"). The large macronucleus (fig. B, *2*, *macron.*) lies a short distance posteriorly from the micronucleus and slightly ventral in position. It, too, lies in the dense anterior portion of the endoplasm.

The *macronucleus* varies from spherical to ovoidal, 15–20μ on its longitudinal axis, and 10–15μ on its transverse axis. The longitudinal axis of the macronucleus is usually nearly perpendicular to the main axis of the body. The nuclear membrane is thin, but stains distinctly with iron haematoxylin. The chromatin material varies widely in appearance in different individuals. In some individuals, small, round granules occupy the whole macronucleus (pl. 11, fig. 1), in other individuals (fig. B, *2*) most of the chromatin, in the form of irregular granules, is concentrated in a single mass with a wide clear area separating it from the nuclear membrane. Irregular branching bits of chromatin, more intensely basophilic than the main chromatin mass, are found in this wide clear space, many of them adhering to the inner surface of the nuclear membrane. We were not able to identify any of the different types of macronuclei as being preparatory stages or reorganization stages attendant upon division, nor does their relative frequency of occurrence give any clue as to whether any one may be considered the typical interphase nucleus. The macronuclear chromatin of dividing individuals (pl. 11, fig. 5) is arranged in relatively large round, lightly staining granules. None of the earlier stages of division was found.

The *micronucleus* is a small spherical or ovoid body from 3μ to 6μ in diameter. The chromatin is concentrated in an irregular mass, separated from the thin nuclear membrane by a narrow, clear space.

NEUROMOTOR SYSTEM

PERIPHERAL CILIA

The short, peripheral cilia are arranged in from 46 to 48 longitudinal rows, extending straight antero-posteriorly. Each row is composed of a series of basal granules (fig. B, *2, bas. grn.*) from each of which a single cilium arises. These granules are spaced about 1–4μ apart, and are connected by a relatively heavy *longitudinal ciliary* fibril (fig. B, *1, l. c. fib.*). A series of delicate transverse strands, the *commissural fibrils* (fig. B, *1, com. fib.*) connect the basal granules of adjacent ciliary rows. A basal granule may give off two commissural fibrils to an adjacent ciliary row, resulting in a V-shaped figure (pl. 11, fig. 2) but this is relatively rare, the typical structure being a regular, rectangular lattice work.

Pickard (1927) describes in the neuromotor system of *Boveria teredinidi,* delicate, wavy, *interstrial fibrils,* running longitudinally between the ciliary rows, connecting the commissural fibrils. No fibrils corresponding to the interstrial fibrils of *Boveria* occur in *Eupoterion,* and in this respect the peripheral fibrillar system is similar to the fibrillar system in *Paramoecium* (Brown, 1930).

Forty to forty-two of the rows of peripheral cilia arise from the heavy, *anterior connective fibril* (pl. 11, fig. 3; fig. B, *1, a. c. fib.*). This fibril is a heavy, deeply staining bar, extending dorso-ventrally across the middle half of the anterior end of the body beneath the pellicle. Four ciliary rows originate from each end of the fibril, and sixteen to seventeen pairs of ciliary rows arise at intervals of 1–2μ along the rest of the anterior fibril. One member of each pair extends along each lateral surface and finally both meet and fuse at the posterior end of the body. For a space of a few microns from the anterior connective fibril, the longitudinal connective fibrils lack basal granules, thus leaving the extreme anterior end of the body devoid of cilia. This can be observed in living material.

Lynch (1929) has described in *Entorhipidium echini* a fretwork of interconnecting fibrils along the anterior edge, the whole complex being termed the *anterior field.* The area included in the anterior field of *Entorhipidium* appears to be homologous to the *anterior connective fibril* of *Eupoterion pernix.*

Six additional rows of peripheral cilia arise along a line connecting the ventral end of the anterior connective fibril and the cytostome. This is the suture line found in many ciliates. The anterior ends of each pair of rows fuse along the suture line and form a V with the apex pointing anteriorly. The members of each pair fuse posteriorly also. These rows of cilia appear to be connected to the rest of the peripheral cilia and to the oral cilia only by the delicate transverse *commissural fibrils.*

PERISTOMIAL CILIA

The long oral cilia, 10–15μ in length, are arranged in four pairs of rows along the oral groove. The anterior ends of each pair fuse in a V-shaped figure with the apex on the suture line. The peristomial rows are concentric to the rows of short peripheral cilia of the body (fig. B, *2, per. cil.*). The four rows of peristomial cilia on the right side of the groove continue posteriorly and describe a semicircle about the cytostome, ending beneath its left surface. All of the peristomial rows of cilia either end directly in the neuromotorium or are closely connected to it by the *tranverse fibril* or by the *longitudinal ciliary fibrils.*

The *neuromotorium* (fig. B, *1, n. mot.*) is a small, narrow triangular mass lying beneath the left surface of the peristome parallel to the long axis of the body. The anterior end of the neuromotorium ends in the heavy *transverse fibril* (fig. B, *1, trn. fib.*) lying at right angles with the main motor mass. The right end of this fibril extends inward and joins the pharyngeal fibrils forming the *pharyngeal strand* (fig. B, *1, ph. str.*) which often runs anteriorly to the region of the macronucleus. This strand and its component fibrils have been described in connection with the cytostome. The left end of the transverse fibril extends away from the peristome and fuses with the two outermost of the peristomial ciliary fibrils and then continues farther to the left, ending in the connective fibril of the adjacent row of short cilia.

The two innermost pairs of peristomial ciliary rows, bordering the oral groove and the cytostome, are conspicuous because of their closely packed line of basal granules and the heavy longitudinal connective fibrils. The basal granules of each peristomial row are much more closely packed than in the ordinary rows of cilia, so close that each granule is almost in contact with the adjacent granules of the row.

The two rows of cilia lying to the right of the mid-line, the *main peristomial rows* (fig. B, *1, m. pst. r.*) proceed posteriorly along the right-hand border of the oral groove and then curve to the left around the posterior border of the cytostome. Here they come closer together and the connective fibrils of the two rows fuse with the posterior end of the neuromotorium.

The two *accessory peristomial rows of cilia* (fig. B, *1, ac. pst. r.*) arise on the suture line from the anterior ends of the *main peristomial rows.* The accessory rows run posteriorly along the left-hand border of the ciliary groove to the cytostome, where their posterior ends bend to the left and proceed a short distance along the lip of the cytostome. These rows are much closer together than any of the other rows of cilia. No fibrils were found connecting the posterior ends of the *accessory peristomial* rows and the neuromotorium. Apparently the only connection is through the connecting fibril of the main peristomial rows.

Two additional rows of heavy peristomial cilia, the *outer peristomial rows* (fig. B, *1, o. pst. r.*) arise from the suture line anterior to the main peristomial rows and proceed parallel to the main peristomial rows and from 6 to 8 microns to the right of them. As they describe a semicircle around the mouth, they gradually approach each other and their connective fibrils fuse and continue anteriorly to meet the transverse fibril as a single strand. Two rows of cilia of the ordinary short peripheral type arise from the anterior ends of the outer peristomial rows and proceed posteriorly to the left of the accessory rows of cilia. The row nearest the oral groove ends abruptly at the transverse fibril. The connective fibril of the other row continues posteriorly beyond the transverse fibril and fuses with the fibril of the adjacent ciliary row of the body. No cilia arise from the portion of the connective fibril posterior to the transverse fibril. These two rows of cilia arising from the anterior ends of the outer peristomial rows are of the ordinary peripheral type occurring over most of the body and are connected with the rest of the ciliary rows of the body by the delicate commissural fibrils, thus uniting the peristomial cilia with the cilia of the rest of the cytosome.

DISCUSSION

All the cilia of *Eupoterion pernix* are integrated by a network of fibrils connecting the basal granules. The neuromotor system is composed of two main parts, the peripheral system of relatively short cilia, and the peristomial system of long, closely spaced cilia. The fibrils of the peripheral system center in the anterior connective fibril, while the peristomial system centers in the neuromotorium which lies beneath the wall of the cytostome. Integration between the longitudinal connective fibrils of the two systems is accomplished by the commissural fibrils running between the various ciliary rows, and by the transverse fibril extending from the neuromotorium. The morphology of this fibrillar system fulfills in every respect the concept of the neuromotor system first developed by Sharp (1914) and extended by many recent workers.

From observations on living material and a consideration of the morphology of the neuromotor system, we conclude that the peripheral cilia are used exclusively for locomotion, while the peristomial cilia, though important in locomotion, are used to produce a current into the cytostome. The oral cilia, because of their greater size and because of the characteristic spiral of the peristomial ciliary rows, tend to impart a spiral movement to the organism as it swims through the intestinal fluid.

The interpretation of the fibrillar system connecting the motor organelles of ciliates as neuroid elements has given rise to considerable controversy. Reichenow (1927, pp. 56, 176–177) has summarized most thoroughly the objections to this hypothesis. He considers these fibrils to be either myonemes or skeletal structures. The coordination of ciliary action, a phenomenon noticed by all students of ciliates, is considered by Reichenow to be accomplished by the whole protoplasm and not by special organelles. Alverdes (1923) found that in a *Paramoecium* artificially constricted across the mid-region, the cilia of each end were still coordinated and each end acted as a single unit. Both Reichenow and Alverdes interpret this as positive proof that the whole protoplasm and not the neuromotorium coordinate the ciliary beat.

Brown (1930) points out that the fibrils connecting the cilia of *Paramoecium* cannot be considered as myonemes. In *Eupoterion pernix,* also, no active change of body form takes place and the myonemal explanation breaks down. The explanation of the fibrils as supporting structures is also inadequate. The fibrils are exceedingly delicate and only an infinitesimal portion of the bulk of the whole ciliate. The pellicle, a tough resistant membrane, distended by the turgidity of the cytoplasm, is sufficient to give a constant body form and at the same time a form which though temporarily deformed by contact with solid objects, will immediately regain its normal shape as soon as this pressure is removed. The fibrils are not necessary to keep the cilia anchored. The insertion of the cilia through the resistant pellicle would more than account for the constant position of the cilia. Alverdes' experiments, which Reichenow considers as final evidence against any neuroid interpretation of the fibrils, can be interpreted just as satisfactorily as a demonstration of the efficiency of the commissural fibrils and as a demonstration that the neuromotorium is primarily a coordinator of the peristomial cilia and only secondarily a coordinator of the body cilia.

Taylor (1920) and MacDougall (1928) give positive experimental evidence supporting the neuromotor hypothesis. Taylor, using *Euplotes,* and MacDougall, using *Chlamydodon,* cut various parts of the neuromotor system of these ciliates by means of micro-dissection apparatus. They found that these operations disturbed or destroyed the coordination of cilia depending upon the importance of the fibrils cut.

Gray (1928) gives a thorough summary of the known facts of ciliary movement and the various theories which have been developed in that connection. In his discussion of the ciliate protozoa (pp. 130–132) he summarizes the morphological and physiological evidence for the neuromotor hypothesis, and concludes: "It seems reasonable to regard these fibres as conductile in function, and as capable of controlling the movements of the cilia, although the latter retain their power of autonomous movement."

SYSTEMATIC POSITION

Eupoterion pernix belongs to the order Holotricha, sub-order Trichostomata. We are using the classification adopted by Reichenow (1929). *Cryptochilum* Maupas and *Conchophthirius* Stein present the closest relationships to this ciliate from the limpet, so we place it with the above two genera in the family *Conchophthiridae* Reichenow. These three genera are alike in that they are flattened laterally, the mouth is conical with a more or less prominent oral groove leading to it, and the peristomial cilia are longer than the cilia of the body. The contractile vacuole in *Cryptochilum* is located posteriorly, in *Conchophthirius* anteriorly or medianly. They are all parasites (commensals?) of mollusks or echinoderms.

Cryptochilum, according to Hentschel, is exceedingly flattened and almost leaf-like, the oral groove is S-shaped, and the cytostome is located anteriorly. Hentschel lists two valid species, *C. echini* Maupas and *C. boreale* Hentschel, both found only in Echinoderms. *Eupoterion,* on the other hand, is only slightly flattened laterally, is ovoid rather than leaf-like, the oral groove is a narrow triangle, and the mouth is located posteriorly. It is found in the stomach content of the mask limpet.

Conchophthirius, according to Reichenow (1929) is strongly flattened laterally, and it crawls on its left surface which is slightly concave. *Eupoterion* shows no tendency to creep on the substrate. The oral groove of *Conchophthirius* is relatively long and the mouth is in the mid-region, thus approaching the situation found in *Eupoterion. Conchophthirius anodontae* has been found only in the mantle cavity of the fresh water mollusks *Anodonta* and *Unio. Conchophthirius* was erected by Stein (1861) for the ciliate *Leucophrys anodontae* described by Ehrenberg (1838).

De Morgan (1925) has summarized the literature on *Conchophthirius.* He recognizes four species: *C. anodontae* (Ehrenberg) Stein from *Anodonta* and *Unio; C. streenstrapii* Stein from *Succinea amphilia; C. curtus* Englemann from *Anodon;* and *C. mytili* De Morgan from the "common salt-water mussel."

Eupoterion gen. nov.

Diagnosis.—Small, ovoid holotrichous ciliates, slightly flattened laterally; short peripheral cilia of the body arranged in longitudinal rows extending straight antero-posteriorly; rows of long oral cilia arise in an oral groove on the mid-ventral surface and extend posteriorly, making a half-turn to the left around the cytostome; small, conical cytostome lies in the postero-ventral edge of the body; contractile vacuole present.

Type species.—*Eupoterion pernix* found in the intestine of *Acmaea persona* Eschscholtz, from the shore along Golden Gate Park, San Francisco, California.

Eupoterion pernix sp. nov.

Diagnosis.—Forty-six to forty-eight rows of short cilia over the body; six rows of heavy peristomial cilia; single contractile vacuole located posteriorly. Length 38–56μ (ten individuals).

Host.—*Acmaea persona* Eschscholtz, swimming freely in the intestinal contents; Golden Gate Park, San Francisco, California.

SUMMARY

1. *Eupterion pernix* gen. nov., sp. nov., found in the intestine of the mask limpet, *Acmaea persona* from Golden Gate Park, San Francisco, California, is described.

2. Observations were made from both living material and permanent mounts.

3. A detailed study of the morphology revealed a neuromotor system which fulfills all the requirements of the neuromotor hypothesis.

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EXPLANATION OF PLATE

PLATE 11

Eupoterion pernix gen. nov., sp. nov., from *Acmaea persona.*

All figures were drawn by aid of camera lucida from individuals fixed in hot Schaudinn's fluid and stained with Heidenhain's iron haematoxylin; × 1500. Many of the cilia are omitted for the sake of clearness and are indicated only by basal granules.

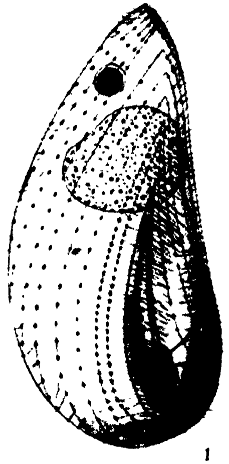
Fig. 1. Ventral view showing the arrangement of peristomial cilia.

Fig. 2. Detailed structure of the fibrils of the ciliary rows covering the body.

Fig. 3. Anterior view showing the anterior connective fibril and the rows of cilia arising from it.

Fig. 4. Dorsal view showing the origin of the ciliary rows of the body from the anterior connective fibril.

Fig. 5. Left lateral view of a late division stage. The nuclear reorganization is nearly complete, the reorganization of the new peristomial region (in the anterior daughter) is almost finished, and plasmotomy is well under way. Many of the ciliary fibrils are breaking along the line of plasmotomy.



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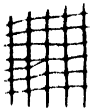

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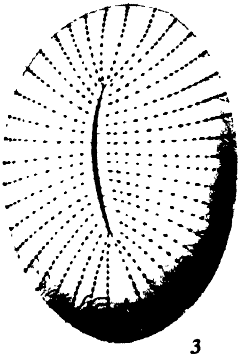

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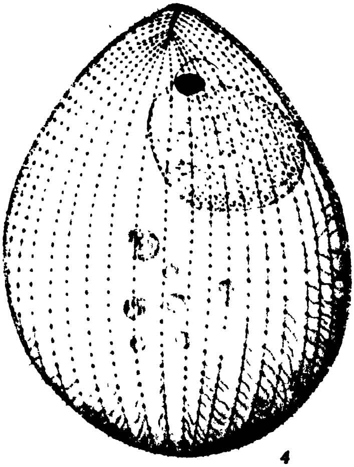

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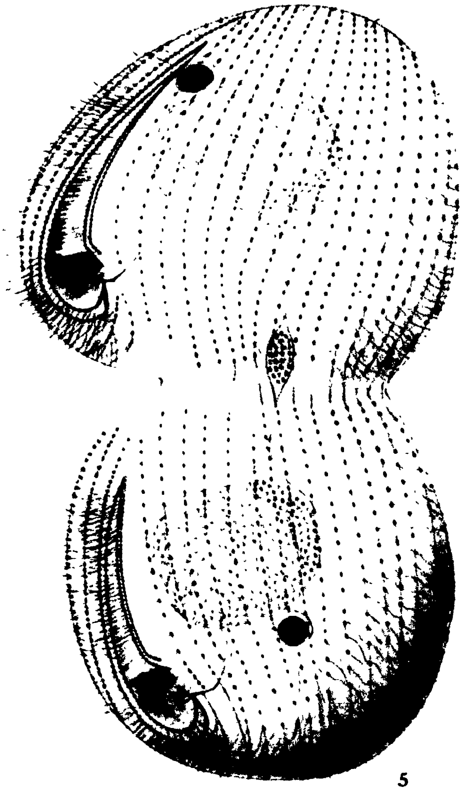

5

SPECIES OF COCCIDIA IN CHICKENS AND QUAIL IN CALIFORNIA

BY

DORA PRIAULX HENRY

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Until the appearance of Tyzzer's monograph on the coccidia of gallinaceous birds, the coccidia in chickens were known as a single species, *Eimeria avium*. The purposes of this paper are to report (1) the occurrence of the four species described by Tyzzer in California chickens, (2) the isolation of three of these species from California quail, and (3) some additional observations on these species.

Materials for these studies were furnished for the most part by Dr. J. R. Beach and Dr. S. Michael, of the Division of Veterinary Science, College of Agriculture. The author also wishes to acknowledge the cooperation of Dr. C. A. Kofoid in the prosecution of this work.

For the experimental work reported in this paper, day-old hatchery chickens were used. Rigid isolation was maintained to prevent accidental coccidial infection, especially after two laboratory infections with *Eimeria acervulina* had occurred. Chicks infected with the different species of coccidia were isolated as well as the available space permitted; those infected with *E. acervulina* and *E. tenella* were kept in separate rooms and those infected with *E. mitis* and *E. maxima*, at opposite ends of a third room. No cross-infection of the latter two was encountered.

Oocysts used in infecting the chickens were allowed to sporulate and were then stored until used in homeopathic vials containing 2.5 per cent potassium bichromate. Later in the work Tyzzer's method of sporulating cysts in Petri dishes was adopted and proved more satisfactory. The infective material was placed directly in the crop of the experimental birds by means of a pipette. With the exception of a few experiments in which a small known number of cysts were used, no attempt was made to estimate the number of oocysts fed.

The method used in most instances to procure pure infections by a single species was as follows: a bird was infected with material containing two or three species and allowed to recover, after which it was fed material containing only a single species in addition to the species already used for immunization. By careful selection of field material, any one of the four species may be obtained pure in a relatively short time.

Although our observations and experimental work are for the most part in accord with Tyzzer's findings, it seems desirable to summarize briefly the chief characteristics of each of the four species and outline the results of our work.

Eimeria maxima Tyzzer

Plate 12, figures 1–5

Eimeria maxima is the largest species of *Eimeria* found in chickens. The oocysts (figs. 1–4) are conical in shape and narrower at one end. The narrower end may be pointed but is usually truncate in appearance. The oocysts vary in length from 22.4μ to 41.6μ and in width from 19.2μ to 25.6μ; the average size is 25.6μ by 22.4μ. They are brown in color and usually have a very irregular, thick cyst wall (fig. 3). The brown color results from an irregular layer of pigment laid down just inside the cyst wall. Near the narrower end of the cyst characteristically one or two globular masses may be seen (figs. 2, 3, and 4). At certain times during the course of an infection in an individual most of the cysts are passed with large amounts of extraneous material adherent to their outer surface. This material is probably cellular fragments from the intestine of the host. Occasionally degenerate cysts are encountered which contain motile bodies. Tyzzer suggests that these bodies may be parasitic microorganisms, but it seems more probable that they are merely products of degeneration. Such cysts invariably fail to sporulate.

The time necessary for complete sporulation of cysts of *E. maxima* when vials are used is three days. Tyzzer does not give the time of sporulation of this species.

The first pure infection of *E. maxima* was obtained by feeding the caecal contents of a chicken which had died twelve hours previously of coccidiosis. This material, which also contained cysts of *E. acervulina* and *E. tenella,* was fed directly to two experimental birds, with no time allowed for sporulation. These infections may be explained by assuming that the caecal contents contained sporulated cysts of *E. maxima.* Fully sporulated cysts of *E. maxima* have been found in the intestinal contents in several other cases.

A pure strain of *Eimeria maxima* obtained from one of the infected birds has been passed successively through seven chickens. After recovery, some of these birds were fed with cysts of the other three species and in all cases they proved susceptible.

The developmental period of *E. maxima* in the chicken is six days from the time of ingestion to the appearance of cysts in the faeces. Schizonts have not been found before the fourth day after feeding. At this time large numbers may be found in sections of the duodenum. On the fifth day of the infection large numbers of the gametocytes may be observed in the subepithelial tissue in the lower parts of the digestive system (fig. 5). Tyzzer believes that *E. maxima* invades only the epithelial cells of the intestine, which migrate to a subepithelial position, carrying their parasites.[1] Cysts have been found throughout the intestinal tract, but in the later stages they are almost invariably found only in the lower part of the intestine. Tyzzer reports *E. maxima* in the wall of the caecum, but we have never been able to demonstrate it in sections of the caecum even when the ileum was heavily infected.

Chickens artificially infected with *E. maxima* frequently become visibly affected but rarely succumb to the infection. They often pass blood, sometimes in rather large quantities, but never as much as in the case of an infection with *E. tenella*. The most characteristic feature of the contents of an infected intestine is the stringy mucoid material which is invariably present. Great masses of cysts are imbedded in this substance.

Chickens ranging in age from three days to eleven months proved susceptible to *E. maxima* infection, but, as in all avian species of coccidia, the younger birds were the most susceptible.

Eimeria acervulina Tyzzer

Plate 13, figures 8–9

Eimeria acervulina is perhaps the most frequently encountered species of coccidia in chickens and, as will be reported later in this paper, is frequently found in California quail. The pathogenicity of *E. acervulina* is relatively slight as compared with that of *E. tenella*. The former, however, is by far the more infective of the two. Because of this, work with any of the other species of coccidia in chickens may be complicated by cross-infections with *E. acervulina* when this species is present in the laboratory. Two such intercurrent infections occurred during the course of this work.

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[1] Personal communication to the author.

The oocysts of *E. acervulina* (fig. 8) found in California vary in length from 12.8μ to 19.2μ, with an average of 16μ, and in width from 12.8μ to 16.0μ. They are narrower in proportion to their length than any of the other *Eimeria* in chickens. The measurements for this species as described by Tyzzer are from 17.7μ to 20.2μ. Sporulation under the conditions described for *E. maxima* ordinarily is completed on the second day in *E. acervulina.* Occasionally completed sporulated cysts may be observed within twenty-four hours.

The prepatent period of *E. acervulina* has been found to be five days in all cases. Tyzzer reports that the oocysts appear promptly at the end of the fourth day.

Eimeria acervulina is encountered in chickens principally in the anterior portion of the small intestine. The most characteristic lesions caused by an infection with this species are grayish white placques on the mucous surface of the small intestine. These areas vary in size from two to six millimeters in diameter and consist of epithelial cells packed with the oocysts of this parasite.

Eimeria tenella Raillet and Lucet

Plate 13, figures 10–14

Eimeria tenella is the most pathogenic of the coccidia found in chickens. This species is the cause of acute coccidiosis, which frequently ends fatally, particularly in young chickens. The oocysts of *E. tenella* (figs. 10–12) found in California birds agree in size exactly with that given by Tyzzer. They vary in length from 19.2μ to 25.6μ and in width from 16.0μ to 22.4μ. In size this species lies between *E. maxima* and *E. acervulina.* The oocysts are broadly conical in shape and the cytoplasm is heavily granular and often in freshly passed faeces rounded up into a sphere in the center of the cyst. This is occasionally seen in freshly passed oocysts of *E. maxima* but has never been seen in *E. acervulina* or *E. mitis.* The distinctly greenish color of the cytoplasm is in strong contrast to the clear, transparent cytoplasm of *E. acervulina.* Complete sporulation occurs in 2 per cent potassium bichromate in two days.

The developmental period of *E. tenella* from the time of ingestion to the passage of newly formed cysts is seven days. This is longer than that of any of the other species in chickens. However, from the fourth or fifth day after the feeding of a large number of cysts the chicken frequently becomes visibly affected and large amounts of blood may be found in the faeces for several days. In fatal infections the bird usually dies on the sixth to eighth day, or may live until about the fourteenth day. If death does not occur at the end of two weeks the bird usually recovers. At necropsy of chickens succumbing to an acute infection of *E. tenella* the caeca are usually found to be distended with blood. In birds killed two weeks or more after infection the caeca contain a hard, cheesy core which is yellow or blood-tinged, and frequently the distal end of the caeca shows hemorrhagic areas. These cores consist to a large extent of solidly packed oocysts. In very severe infections the lower part of the small intestine and the large intestine are also invaded and hemorrhages may likewise occur in these regions.

Eimeria mitis Tyzzer

Plate 13, figures 6 and 7

Eimeria mitis is the smallest of the *Eimeria* of chickens. The oocysts (figs. 6, 7) of this species are quite easily distinguished from the oocysts of the other species by their small size and characteristic appearance. They are usually almost spherical in form and the cyst wall is not easily distinguished, as the cytoplasm is applied very closely to its inner surface. According to Tyzzer, the length varies from 14.3μ to 19.6μ, average, 16.2μ. No pure infection with *Eimeria mitis* from chickens was established in chickens, although mixed infections with *E. maxima* were established.

Eimeria mitis was obtained in a pure culture from a chicken infected by material containing *E. mitis* and *E. tenella* from the California quail. Faecal material containing cysts collected on the fifth and sixth day after feeding the caecal contents of the quail was allowed to sporulate and then fed to another chicken. Four and one-half days later cysts of *E. mitis* were recovered and subsequent examinations indicated a pure infection with *E. mitis.* In successive transfers through other chickens cysts were also recovered four and a half days after feeding. In the experiments of Tyzzer the prepatent period of *E. mitis* was found to be five days.

Cysts of *E. mitis* may be found along the whole length of the small intestine, but are commonly found in the upper or middle portion. In heavy infections with *E. mitis,* characteristically a grayish mucoid substance containing enormous numbers of oocysts appears

TABLE I

SUMMARY OF THE DISTINGUISHING CHARACTERISTICS OF THE SPECIES OF COCCIDIA FOUND IN CHICKENS

	Size of occysts Length Width	Form	Color of protoplasm	Sporulation time Tyzzer	Prepatent period	Usual position in intestine	Pathology
Eimeria tenella	19.6–26.1μ 16.3–22.8μ Average 22.6x19.0μ	ovoid	greenish	48 hours	7 days	Caecum	Hemorrhage
E. maxima	21.4–42.5μ 16.5–29.8μ Average 29.3x22.6μ	egg-shaped, pointed at one end	brown	not given (in vials, 72 hours)	6 days	Middle and lower part of intestine	Grayish exudate; may be blood-tinged
E. acervulina	17.7–20.2μ 13.7–16.3μ (Tyzzer) 12.8–19.2μ (Henry) Average 19.5x14.3μ	egg-shaped, narrow	clear	20 hours	4 days (Tyzzer) 5 days (Henry)	Upper part of intestine	Whitish or gray spots on surface of mucosa
***E. mitis* in chicken**	14.3–19.6μ 13.0–17.0μ (Tyzzer) Average 16.2x15.5μ	nearly spherical	clear	48 hours	5 days	Upper part of intestine	None (Tyzzer)
in chicken from quail	9.6–16.0μ 9.6–16.0μ Average 12.8x11.2μ	nearly spherical	clear	48 hours	4½ days	Upper part	Mucus, sometimes blood-tinged

in the faeces. In some cases this exudate is blood-tinged. A three-weeks-old chicken which was fed massive doses of *E. mitis* on three consecutive days died on the eighth day after the initial feeding. Blood was passed for three days previous to death and on the seventh day the chicken was droopy and ate little food. At autopsy no lesions were noted, but blood-tinged mucus and large numbers of cysts were found in the small intestine. Several other young chickens became sick when fed large doses, but recovered. This differs from Tyzzer's findings with *E. mitis* from chickens in that massive doses had no effect on the chickens and blood-tinged mucus was not found in the intestinal contents.

As an aid to the ready recognition of the four species of *Eimeria* found in chickens, table 1 presents the most important similarities and differences in morphology, pathology, and life history of the various species.

EXPERIMENTAL DATA

During the course of the work of separating and studying the several species of *Eimeria* of chickens, several interesting although unrelated observations were made and methods perfected which may be of value in the study of the species of coccidia in other animals as well as those found in chickens.

In an attempt to determine the optimum concentration of potassium bichromate for the sporulation of the cysts of *Eimeria maxima,* tests were made of solutions containing varying amounts of potassium bichromate. One, 2.5, and 5 per cent concentrations were used. No difference was found in the sporulation time of the cysts in the two lower dilutions. The 5 per cent solution, however, definitely retarded this process. As the 1 per cent solution is not sufficient to prevent the rapid growth of many contaminants, the 2.5 per cent solution has been chosen for use for routine storing of the oocysts of a large number of species of *Eimeria* as well as several species of the genus *Isospora.* This solution has prevented serious contamination in most cases and has allowed sporulation to occur in all species.

That potassium bichromate in concentration of 2.5 per cent has little or no injurious effect upon coccidial cysts has been amply demonstrated by experiments in which birds have been infected with cysts which have remained in this solution over a long period of time. Oocysts of *Eimeria maxima* have been proved to be infective for chickens after six or seven months' storage in 2.5 per cent potassium bichromate. In one instance cysts of the same species which had been kept for eleven months failed to infect a chicken which was later proved to be susceptible to a more recently obtained strain of the same species.

It has been found desirable on several occasions to use cysts freed entirely of living bacteria or other microorganisms. A simple method for obtaining sterile cysts is as follows: faeces containing the cysts are suspended in normal salt solution and strained through a fine mesh screen. The strained material is centrifuged and the sediment containing the majority of the cysts placed in a sterile tube. Full strength hexyl resorcinal is added. However, as there is some liquid present in the material containing the cysts the final dilution is approximately one-half strength. At the end of fifteen minutes the liquid is diluted with several volumes of sterile saline and again centrifuged. After the supernatant fluid has been discarded cysts may be stored until sporulation occurs. Cultures made from the material treated in this manner upon suitable bacteriological media remained sterile. Sporulated oocysts may be treated in a manner similar to that described for unsporulated cysts. However, in order to prove that such cysts are not injured by the treatment, it is necessary to infect animals with them. In this work no treated cysts failed to produce infection.

Pure infections of a single species were obtained in two ways, (1) by building up an immunity and subsequent infection with a mixed culture, and (2) by feeding one or a very few cysts. As a practical method the former is the more useful. It is of interest, however, to determine the minimum infective dose. Infection has been secured by the introduction of a single oocyst into a young chick. The species used in this case was *E. maxima.* Three other birds which also received a single oocyst did not become infected. As Tyzzer found in similarly inoculated animals, oocysts were not passed until the seventh day, as contrasted with a prepatent period of six days for a heavy infection, and the infection persisted for only three days. Another bird was successfully infected with six oocysts of *Eimeria maxima.* As in the case of the chicken infected with a single cyst, the first cysts appeared in the faeces on the seventh day and persisted for a total of only three days. In this case sufficient infection had apparently occurred to produce definite lesions, as the faeces contained some blood. Ten days after the last cysts were found in this bird a massive dose of *E. maxima* produced infection. The small number of cysts used in the initial infection had apparently not produced an immunity to the same extent as that produced by larger numbers of cysts in other birds.

During the course of these experiments coccidia were obtained from a miscellaneous group of birds and mammals. After sporulation of these oocysts, an attempt was made to infect two- to four-weeks-old chicks with these several species. The species used which failed to infects chickens were the following: *Eimeria debliecki* and *Eimeria scabra* from the pig, *Eimeria stiedae*, *E. perforans*, and possibly others from the rabbit, *Eimeria zürni* and *E. smithi* from the cow, *Eimeria caviae* from the guinea pig, and several undescribed species of *Eimeria* from small mammals, as well as *Isospora lacazei* from birds. The failure to infect chickens with *Eimeria* from swine is not in accord with the findings of Biester and Murray (1929), who were able to find oocysts in the faeces of chickens after feeding material containing *Eimeria* from swine. As Biester and Murray recognized only one species in hogs, it cannot be determined which species they used. Henry (1931) has found four species in swine. Likewise Uhlhorn (1926) produced infections in chickens with oocysts from the rabbit. As in the case of the pig, the several species found in the rabbit were not recognized at the time of this work. Kessel (1930) has shown that there are five species in the domestic rabbit.

Successful infection of chickens was obtained by feeding *Eimeria* oocysts from two turkeys which had succumbed to coccidiosis. Morphologically the oocysts (pl. 13, figs. 9, 13) from these two cases were identical with oocysts of *E. acervulina* and *E. tenella* as found in chickens. The lesions produced in the first chickens infected, as well as in subsequent infections after the first passage, resembled typical coccidiosis of chickens. The possibility of these experimentally infected birds having become infected by any means other than the cysts fed can be almost completely ruled out because of the ideal conditions under which the experiment was carried on. The birds were kept on an upper floor of a new building in which no animals had previously been housed and no normal or infected birds other than those used in the experiment were present during the course of the work. Control birds showed no signs of infection.

The conditions outlined above have been emphasized because Johnson (1923) was unable to produce cross-infection with the *Eimeria* found in chickens and turkeys. Tyzzer (1929), on the basis of Johnson's work, in addition to several unsuccessful attempts to produce cross-infections in his own laboratory, has described two species of *Eimeria* found in turkeys, *Eimeria meleagridis* Tyzzer (1927) and *E. meleagrimitis* Tyzzer (1929). *Eimeria meleagridis* (pl. 13, fig. 15) has also been seen in turkeys in California.

COCCIDIA OF THE CALIFORNIA QUAIL

Tyzzer has described a new species, *Eimeria dispersa*, from the Eastern Quail, or "Bobwhite," *Colinus virginianus virginianus.* Other than this species no coccidia were found by this author. In cross-infection experiments Tyzzer was uniformly successful in infecting turkeys with *E. dispersa* and occasionally able to establish slight infections in chickens.

California quail (*Lophortyx californica vallicola* and *Oreortyx picta plumifera*) from game farms have been examined from time to time through the courtesy of Dr. E. C. O'Roke. In addition to these a few wild quail have been examined.

Three species of coccidia have been found in caecal and intestinal contents of the California quail. Two of these species are apparently morphologically and physiologically identical with two of the species found in chickens, namely *Eimeria tenella* (pl. 13, fig. 14) and *Eimeria acervulina*. The third species closely resembles *E. mitis*, but differs in being somewhat more pathogenic for chickens than *E. mitis* described by Tyzzer. Unfortunately at the time this paper was written no oocysts of the latter species were available for microphotographs.

The quail strains of these three species have been obtained in pure culture by passage through chickens, and characteristic infections produced in further inoculations.

As the quail examined were in most cases birds which were in good health, and as no experimental infections have been attempted with quail, the effect of massive doses upon quail has not been determined. However, one dead bird was examined which showed lesions macroscopically identical with those found in typical *E. tenella* infections in chickens. The cysts of *E. tenella* and *E. acervulina* were found in large numbers in the caecal and intestinal contents of this bird.

SUMMARY

1. Four species of *Eimeria* in chickens described by Tyzzer (1929) are reported from California.

2. The occurrence of two of these species, *E. acervulina* and *E. tenella* in turkeys, and of these two species and *E. mitis* in quail is reported.

3. The failure to infect chickens with various coccidia from other hosts is also noted.

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EXPLANATION OF PLATES

Photomicrographs by J. E. Gullberg.
Magnification, figs. 1–4, × 1600.

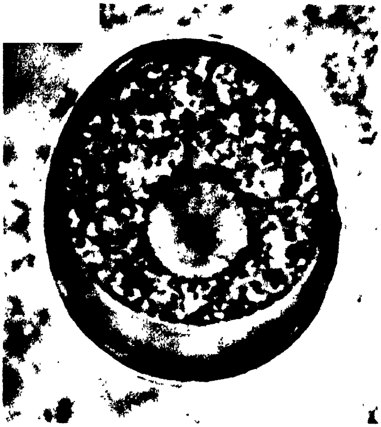
PLATE 12

Figs. 1–5. ***Eimeria maxima* Tyzzer.**

Fig. 1. Non-sporulated cyst. Cytoplasm beginning to recede from cyst wall.

Figs. 2–4. Sporulated cysts. Polar granule showing at one end. In figure 3 is shown a cyst with a slightly roughened cyst wall.

Fig. 5. A section of the ileum of a chicken showing macrogametes of *E. maxima* lying in the tunica propria. Tissue fixed in Zenker's and stained in iron haematoxylin. × 275.



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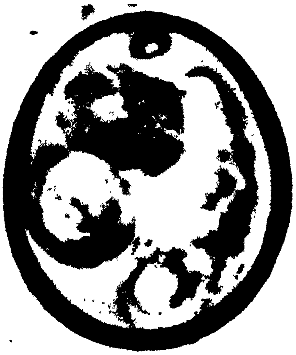

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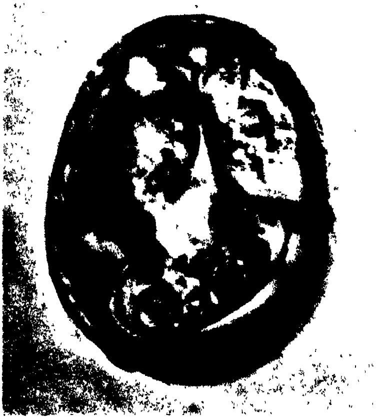

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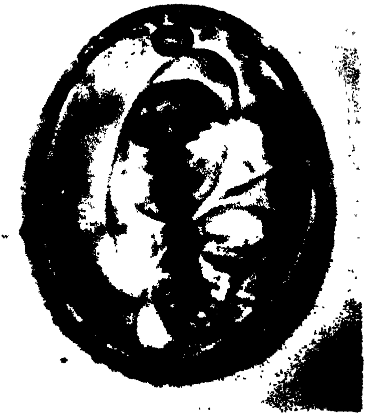

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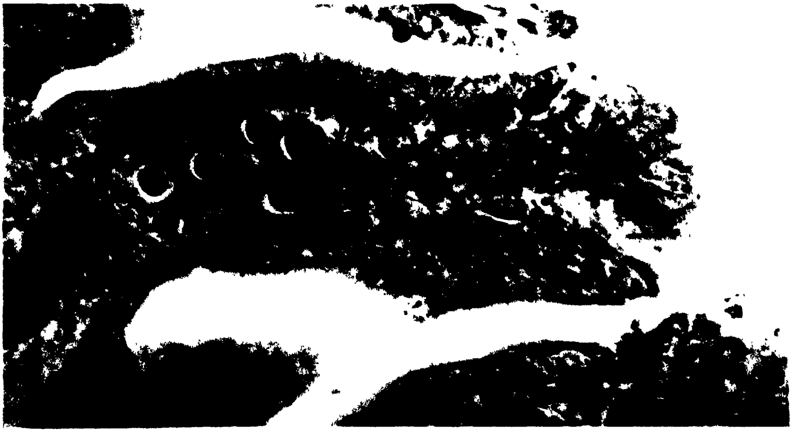

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PLATE 13

Magnification × 1600.

Figs. 6–7. *Eimeria mitis* Tyzzer.

Fig. 6. Non-sporulated oocyst. Cytoplasm beginning to contract.

Fig. 7. Fully sporulated oocyst.

Figs. 8–9. *Eimeria acervulina* Tyzzer.

Fig. 8. Sporulated oocyst from the chicken. One end of cyst slightly collapsed.

Fig. 9. *E. acervulina* cyst from the intestinal contents of a turkey.

Figs. 10–14. *Eimeria tenella* Raillet and Lucet.

Figs. 10–12. Oocysts of *E. tenella* from the caecal contents of a chick.

Fig. 10. Cyst with cytoplasm beginning to contract.

Fig. 11. Oocyst in first stage of sporulation. Three sporoblasts and part of the fourth in focus.

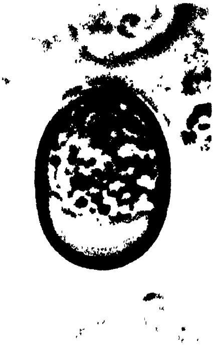
Fig. 12. Fully sporulated cyst.

Fig. 13. Mature cyst of *E. tenella* from the turkey.

Fig. 14. Fully sporulated cyst from caecal contents of quail. The cyst has collapsed in several places.

Fig. 15. *Eimeria meleagridis* Tyzzer.

Fig. 15. Mature oocyst of *E. meleagridis* from the turkey.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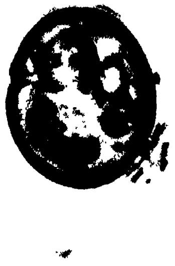
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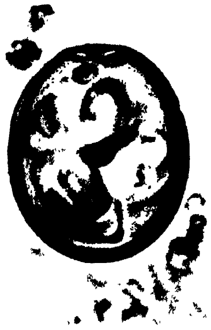
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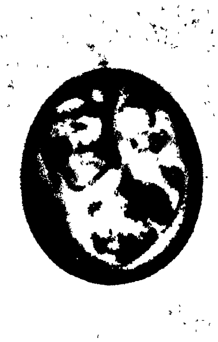
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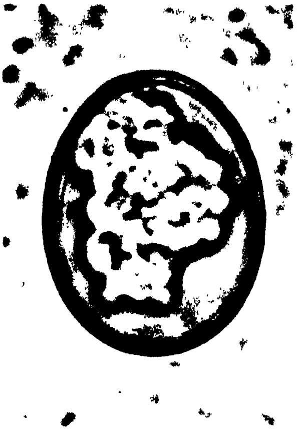
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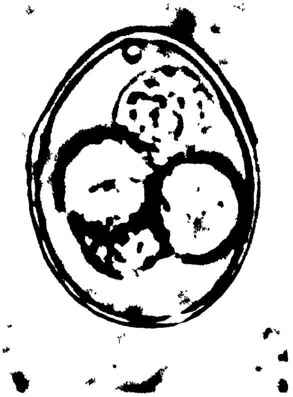
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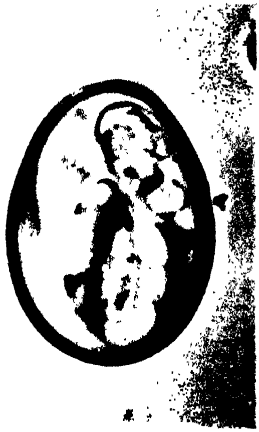
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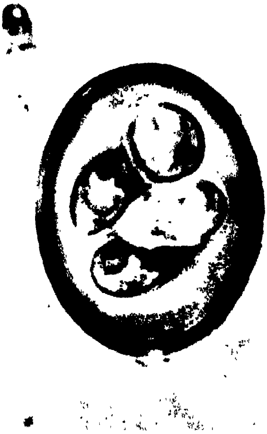
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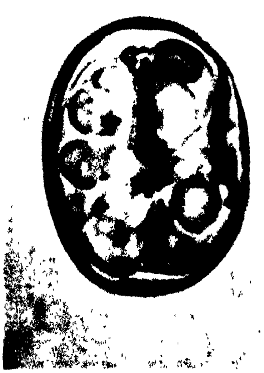
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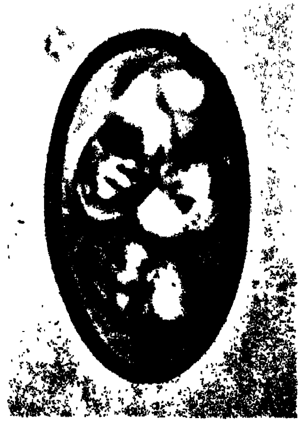
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TRICHOMONAD FLAGELLATES FROM TERMITES

II. EUTRICHOMASTIX, AND THE SUBFAMILY TRICHOMONADINAE

BY

HAROLD KIRBY, Jr.

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GENERAL INTRODUCTION

In the first part of this study (Kirby, 1930) flagellates of the family Trichomonadidae, in the genera *Tricercomitus* Kirby and *Hexamastix* Alexeieff, were described. Members of the former genus were found in *Termopsis* and in all termites of the subfamily Kalotermitinae which were studied, except *Porotermes*. Five new species of *Hexamastix* were recorded from *Termopsis* and seven Kalotermitinae. *Tricercomitus* is known only from termites, though there are flagellates which are more or less closely related in certain vertebrates. *Hexamastix* is represented in other insects and in amphibia.

Eutrichomastix, which appears to be uncommon in termites, is abundantly represented elsewhere, having been reported from vertebrates of all classes, trichopteran larvae, and beetles. The devescovinid flagellates, from *Devescovina* to *Gigantomonas,* obviously have developed from the *Eutrichomastix* type. For the devescovinid flagellates it is convenient to recognize the subfamily Devescovininae in the family Trichomonadidae, instead of the family Devescovinidae Poche, 1913.

Trichomonas and related flagellates with two to five anterior flagella and an undulating membrane are widespread in animals. For these it is convenient to recognize the subfamily Trichomonadinae. Members of this subfamily have been recorded from vertebrates of all classes, leeches, a mollusk, and other insects, as well as from termites. Though found in termites of all the major groups, their distribution in the genera is uneven. They may occur in one species of a genus and not in a closely related species. With *Eutrichomastix,* they are the only flagellates so far found in the Termitidae, where they are often associated with amoebae (Kirby, 1927). The genus *Tricercomitus* also is included in this subfamily.

In this paper brief reviews will be given of the described species of Trichomonadinae from termites, exclusive of *Tricercomitus,* and a number of new species, including one new genus, *Pentatrichomonoides,* will be described. In connection with the study of new and described species, it has been possible to make some additions to the knowledge of the cytology of these flagellates.

The sources of material, with acknowledgments for the generous aid given the writer by students of termites, have been given in the previous paper or are mentioned in connection with the specific accounts. Many of the figures were drawn by Miss Dorothy G. Harris, of whose skill and care the writer makes grateful acknowledgment; some were prepared by the writer.

Eutrichomastix axostylis sp. nov.

Plate 14, figures 1–5; figure C, 3

Host.—*Nasutitermes* (*Subulitermes*) *kirbyi* Snyder. Canal Zone.

From *Hodotermes murgabicus* Vas. of Turkestan Bernstein (1928) described *Eutrichomastix termitis.* This flagellate, which was present in large numbers, was 8–12μ in length, and possessed a short, rod-formed parabasal body, a moderately developed axostyle, a cytostome, and other structures characteristic of *Eutrichomastix.* A species which is undoubtedly different from this and other described species of *Eutrichomastix* occurs in the gut of *Nasutitermes* (*Subulitermes*) *kirbyi* Snyder collected at Barro Colorado Island, Canal Zone. In this termite no other Protozoa were found. The other Protozoa found in *Hodotermes murgabicus* are listed under *Trichomonas vermiformis* (p. 181).

The body of the flagellate is elongate ellipsoidal in form (fig. C, 3, p. 198), sometimes almost cylindrical (pl. 14, fig. 4). Occasionally it is pyriform, narrowing posteriorly. Fifty individuals ranged in length from 5–10.5μ, in breadth from 2–3.5 (4.5 in one exceptional case), averaging $8.1 \times 2.7\mu$. The projecting part of the axostyle constitutes from .5 to 2 (rarely 3) μ, averaging 1.4μ, of this length. The three

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For Figure C, see p. 198.

anterior flagella, which in the preparations were usually closely united into a whip, are about 7.5 (5–10) μ long. The trailing flagellum, which is not stouter than an anterior flagellum, is about 13 (10–16) μ long.

The nucleus is ellipsoidal, very close to the anterior end of the body, and measures about 1–1½ × 1½–2μ. There is a distinct nuclear membrane and a large, granular chromatin mass occupying most of the interior. The anterior end of the nucleus is usually in contact with the blepharoplast, which is a single, moderately large granule. A rather small cytostome, more rounded in form than crescentic, as is usual, is present (pl. 14, fig. 3). In the neighborhood of the nucleus and blepharoplast there are often a number of small siderophile granules in the cytoplasm. Unfortunately, all available material had been fixed in Schaudinn's fluid and stained in iron-alum haematoxylin, so that no parabasal body could be found.

The axostyle is unusually stout. It extends from the region of the blepharoplast along one side of the nucleus, straight through the body, and projects beyond the posterior end. It is of even diameter until close to the end, where it enlarges and then tapers rather abruptly. After tapering to a point it continues for about one micron in a filamentous prolongation. The axostylar membrane shows distinctly in optical section, much more so than does the margin of the body. No endoaxostylar granules have been observed. The axostyle does not meet the posterior end of the nucleus, as is the case with the devescovinids, but passes to one side of the nucleus toward the blepharoplast.

Around the posterior portion of the axostyle, usually just anterior to the point of greatest enlargement, are two rings which in optical section appear as granules close to the axostyle. These usually lie in the cytosome before the axostyle projects, but in case the axostyle projects farther than usual these are carried with it (pl. 14, fig. 2). They vary in degree of development. Frequently they are very conspicuous, deeply stained even when the rest of the body is much destained. In other cases they are smaller, and in some they are not visible at all. In a few cases, only one ring is visible.

Most species of *Eutrichomastix* have slender axostyles. In *E. gracilis* from the caecum of birds it is a slender, stainable filament (Cunha and Muniz, 1927). *E. axostylis* is remarkable in that the axostyle is very stout. In this respect it resembles *E. globosus* as described by Cunha and Muniz (1925). This flagellate, which is smaller than *E. axostylis,* has "well developed siderophile granules" at the point where the axostyle emerges from the cytoplasm, probably sections of one or more periaxostylar rings.

FLAGELLATES OF THE SUBFAMILY TRICHOMONADINAE

INTRODUCTION

The known flagellates of this subfamily are characterized by the possession of from two to five anterior flagella, an undulating membrane at the base of which is a costa or chromatic basal rod, and an axostyle. A parabasal body is a frequent and probably universal structure. Eight named and two unnamed species have been described from eleven termites; one of the named species, *T. dogieli,* is doubtful. In the present account seven new species will be added from eight other termites, and two of the previously described species will be reinvestigated.

In the nomenclature of the organelles of the Trichomonadinae, the rod-like structure beneath the undulating membrane has been called the costa (Kunstler), côte, chromatic basis, chromatic basal rod, and even parabasal body (Kofoid and Swezy, Faust). It seems to the writer that costa is the best term because of its brevity, its convenience in adjective form in costal and in paracostal granules, and in its comparative use in connection with the term cresta for the structure of the Devescovininae which is homologous but not exactly the same. The term blepharoplast has been used in the following account for the granule or one of the distinctly separate granules from which the flagella and other organelles arise, and basal granule for one of the several granules into which the blepharoplasts of some species may be resolved.

There has been much disagreement concerning the splitting of the old genus *Trichomonas* according to the number of anterior flagella. The number of flagella is an important diagnostic character in flagellates, and it is constant in a species except at times preceding or following multiplication. It is used as the principal character to separate genera in various groups (chrysomonads, *Chlamydomonas-Carteria, Euglena-Eutreptia, Astasia-Distigma, Peranema-Heteronema,* the Protomastigida, and others). In the case of the Trichomonadidae also, it seems that consistency demands the recognition of separate genera on the basis of flagellar number. When the flagella are very many, as in the Hypermastigida, it is, of course, not possible to follow this principle.

In the following review, the species are arranged in the order in which they were described.

REVIEW OF PREVIOUSLY DESCRIBED SPECIES FROM TERMITES

Trichomonas termitidis Dogiel, 1916

Figure A, 2

Host.—Termites from near Entebbe, Uganda.

Body elongated, length about 14–18μ, size very variable. Four anterior flagella not quite equal in length. Costa slender. Undulating membrane rather high, forming a semicircle around the body, terminating in a short free flagellum. Probably two basal granules for flagella, only one clearly observed. Cytostome not observed. Axostyle long, rather thick, often protruding posteriorly. Anterior part believed to surround the nucleus. Nucleus oval, distinct membrane, small granules of chromatin scattered within.

Dogiel states that the flagellate occurs in large numbers in its host, and is the only protozoan which he found. The hosts were not very large, dark termites taken from a high, black, mushroom-shaped nest. He says that this was probably a species of *Rhinotermes*. The type of nest suggests one of the Termitidae, rather than *Rhinotermes*. All termites of the family Rhinotermitidae which have been examined contain hypermastigotes in large numbers. *Pseudotrichonympha* has been described from two species of *Rhinotermes*, and Dr. S. F. Light informed the writer that he has seen it in all of several species of the genus which he examined. It seems likely that Dogiel may have had one of the Termitidae. *Cubitermes* builds high, black, mushroom-shaped nests. A *Trichomonas* somewhat similar to *T. termitidis* the writer has found in abundance in *Orthognathotermes wheeleri*, one of the Termitidae, and it was the only protozoan present.

Trichomonas macrostoma Dogiel, 1916

Figure A, 3

Host.—*Hodotermes mossambicus* Hagen. British East Africa.

Shape as in *T. termitidis*, size smaller, 12–15μ long. Axostyle, flagella, blepharoplast as in preceding species. Costa slender. Undulating membrane straighter, free end very short. Nucleus with numerous chromatin granules, and sometimes one or two large chromatin lumps. Cytostome large, deep, extended into a prominent lip within which there often are present two to three food bodies. Vacuoles in body often containing cocci. Rounded stages without flagella found; supposed by Dogiel to be forming cysts.

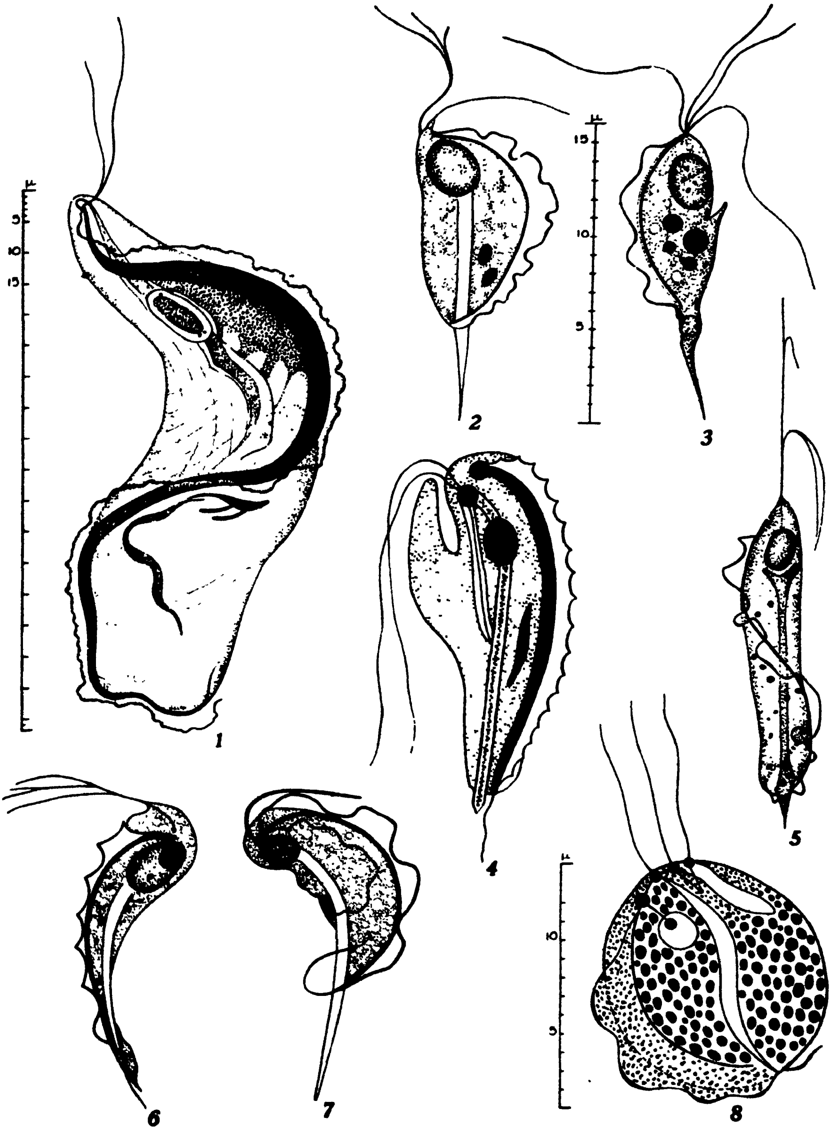

Fig. A. 1. ***Pseudotrypanosoma giganteum*** Grassi (after Grassi, 1917). 2. ***Trichomonas termitidis*** Dogiel. 3. ***Trichomonas macrostoma*** Dogiel (2 and 3 after Dogiel, 1916). 4. ***Ditrichomonas termitis*** Cutler (after Cutler, 1919). 5. ***Trichomonas vermiformis*** Bernstein (after Bernstein, 1928). 6, 7. ***Trichomonas trypanoides*** Dub. and Gras. (after Duboscq and Grassé, 1924). 8. ***Tritrichomonas*** sp. (Mello) (after Mello, 1923). 1 and 4 × 833; others × 2500.

Found in all specimens of *Hodotermes mossambicus* examined. The other Protozoa described by Dogiel from this termite are *Gigantomonas herculea, Myxomonas polymorpha* (which is probably a synonym of *Gigantomonas*), *Joenia intermedia, Holomastigotes elongatum, Holomastigotes crassum, Spirotrichonympha africana,* and *Spirotrichonympha simplex* (which, according to Bernstein, 1928, is a synonym of *Microspironympha porteri* Koidzumi).

Pseudotrypanosoma giganteum Grassi, 1917

Figure A, 1

Host.—Porotermes adamsoni (Froggatt). Australia.

This flagellate is undoubtedly a trichomonad, and does not belong in the Pyrsonymphidae (=Dinenymphidae) to which Grassi (1917) assigned it. Shape variable with degree of contraction, from an elongated trypanosome-like form to one almost circular in outline. Length (from Grassi's figures; measurements not stated) 55–111μ. No cytostome described. Two short, delicate, anterior flagella; low undulating membrane. Costa unusually stout. Several anterior basal granules. No axostyle described, except for the filament doubtfully compared by Grassi to the axostyle of *Dinenympha*. Nucleus at a certain distance from the anterior end, greater than in most trichomonads. Cytoplasm containing a large number of small granules, especially in the region just beneath the costa. Surface of body traversed by a large number of oblique striations.

This organism has been found in *Porotermes grandis* Holmgren, from which material was sent to the writer by Mr. Gerald F. Hill. An account of it is given below (p. 210).

The other species of Protozoa described by Grassi from *Porotermes adamsoni* are: *Joenina pulchella, Spirotrichonympha mirabilis, Spirotrichonymphella pudibunda* and *Trichonympha magna*.

Ditrichomonas termitis Cutler, 1919

Figure A, 4

Trichomonas termitis Imms, 1919; *Trichomonas immsi* Duboscq and Grassé, 1924; *Ditrichomonas immsi* Duboscq and Grassé, 1925.

Host.—Archotermopsis wroughtoni Desneux. India.

This flagellate was described by Cutler in April, 1919. Imms included a description of it under the name *Trichomonas termitis* in his paper on *Archotermopsis* published in September, 1919. The name *Trichomonas immsi* was proposed for it by Duboscq and Grassé (1924) because they believed *termitis* to be preoccupied by "*Trichomonas* *termitis* Dogiel, 1916." Later (Duboscq and Grassé, 1925; Grassé, 1926) they used the designation *Ditrichomonas immsi* (Dub. and Gras.). The name used by Dogiel, however, was *Trichomonas termitidis,* so that *termitis* was not preoccupied.

Average size given by Cutler 50 × 22μ; range given by Imms 30–88 × 13–57μ. Cytostome large. Two anterior flagella about the length of the body; undulating membrane moderately well developed; costa fairly stout. Two blepharoplasts shown. Parabasal body half or more of body length, almost straight, with a parabasal thread through the middle. Axostyle moderately stout, projecting and pointed posteriorly, small granules in linear series within. Nucleus oval, 6–8μ long. Rod-like bodies, usually around anterior part of axostyle, possibly mitochondria. Chromatic blocks (paracostal granules) often present under costa, especially in dividing animals. Centriole emerges from blepharoplast at mitosis, divides to form a moderately stout paradesmose. Parabasal body and axostyle probably divide. Four to seven chromosomes.

The other species of Protozoa described from this termite by Cutler are *Joenopsis cephalotricha*, *Joenopsis polytricha*, *Microjoenia axostylis,* and *Pseudotrichonympha pristina*.

Trichomonas dogieli Duboscq and Grassé, 1923

Host.—Kalotermes flavicollis (Fab.). France.

This species was later (1924) dropped as a synonym of *Janickiella grassii* Duboscq and Grassé, 1923.

Three anterior flagella; rod-like parabasal body; axostyle well developed; abundant frontal granules; slender costa shown in figure; trailing flagellum attached, but no distinct membrane shown in figure; long free portion of trailing flagellum.

This is a form of uncertain status. According to the description, it has all the characters of the subfamily Trichomonadinae, but it appears not to be a true *Tritrichomonas*. It has been shown (Kirby, 1930) that the "*Trimitus*-forms" placed in the life-history of *Janickiella* belong to a separate species, *Tricercomitus divergens*. It may be that this *Trichomonas-form* is also a separate species, in which case it appears to belong in the Trichomonadinae. If it is a stage of *Janickiella grassii,* there seems to be a transition between *Eutrichomastix* and *Tritrichomonas* forms in that species.

Tritrichomonas sp. (Mello, 1923)

Figure A, 8

Hosts.—"Two species of termites from Benguella."

Pyriform or rounded, 11–14 × 4–8μ in former, 5–7μ in latter. Cytostome present. Three anterior flagella, 17–19μ long; moderately developed undulating membrane. Costa very slender. Two blepharoplasts, one composed of three granules, interconnected by rhizoplasts. "Parabasal granule" close to and connected with two of these. Axostyle moderately stout, pointed posteriorly. Nucleus spherical, 2.5–3μ in diameter. Four chromosomes. Rather slender paradesmose.

No other Protozoa have been recorded from these hosts.

Trichomonas trypanoides Duboscq and Grassé, 1924

Figure A, 6–7

Host.—*Reticulitermes lucifugus* Rossi. France.

Form like that of trypanosome, laterally compressed; or more rounded and discoid. Length about 16μ. Cytostome not described. Anterior flagella two to four, number described as variable (?). Undulating membrane well developed. Costa moderately stout. Blepharoplast large. Axostyle stout, frequently projecting more or less from body. Nucleus globular, close to blepharoplast. Large chromatic blocks often present under costa; mass of siderophile granules around axostyle back of nucleus, possibly mitochondria.

Besides *Trichomonas trypanoides,* the following valid species of Protozoa have been described from this host: *Pyrsonympha vacuolata* (Comes) *Dinenympha gracilis Leidy, Hexamastix termitis* (Grassi) Alexeieff, *Spirotrichonympha flagellata* Grassi, *Microjoenia hexamitoides* Grassi, *Trichonympha agilis* Leidy, *Holomastigotes elongatum* Grassi, *Duboscquia legeri* Perez.

Trichomonas termopsidis Cleveland, 1925

Figure B

Trichomitus termitidis Kofoid and Swezy, 1919.

Hosts.—*Termopsis angusticollis* Hagen. California, Oregon. *Termopsis nevadensis* Hagen. California, Oregon.

This flagellate was first described by Kofoid and Swezy (1919). Cleveland (1925) pointed out the presence of an axostyle and four flagella, and Andrews (1925) redescribed the organism including these points and the parabasal body. No trichomonad with three anterior flagella and without an axostyle is present in these termites, so that *Trichomitus termitidis* becomes a synonym of *Trichomonas termopsidis.*

Form pyriform to ellipsoidal or subspheroidal. Length 11–150μ or more; average size given by Andrews, excluding large dividing individuals, 29 × 14.5μ. Cytostome large, elongated. Four anterior flagella, often equalling body in length. Undulating membrane large, free portion of bordering flagellum long. Costa stout. Blepharoplast single, fairly large. Parabasal body long, exceeding half the body length, parabasal thread along one side. Axostyle stout, posteriorly tapering abruptly to a point, usually projecting more or less from the body. Small chromatic granules in linear series in axostyle. Nucleus ellipsoidal, at short distance from blepharoplast, connected by rhizoplast. Centrosome separates from the centroblepharoplast in mitosis, as in *Ditrichomonas termitis.* Moderately stout paradesmose. Chromosomes about twelve.

The other Protozoa of these termites are *Trichonympha campanula* Kofoid and Swezy, *Streblomastix strix* Kofoid and Swezy, *Leidyopsis sphaerica* Kofoid and Swezy, *Hexamastix termopsidis* Kirby, *Tricercomitus termopsidis* Kirby, and a species of gregarine, which is rarely found.

In size and in most structural features this flagellate resembles *Ditrichomonas termitis* closely. The main differences are in the number of flagella and in the blepharoplasts. The division process is exactly comparable in the two, and by comparison of the figures the chromosomes seem similar in form and number.

Trichomonas vermiformis Bernstein, 1928

Figure A, 5

Host.—*Hodotermes murgabicus* Vas. Turkestan.

Elongated, length 15 to 20μ. Cytostome slit-like. Four anterior flagella somewhat shorter than the body. Undulating membrane well developed. Costa rather slender (in figures). Single, rather small blepharoplast. Parabasal body not described. Axostyle rather stout, broadened behind nucleus, enlarged considerably at posterior end, projecting, pointed.

Besides this trichomonad, Bernstein described from this host the following new species: *Trichonympha turkestanica, Holomastigotoides cingulatum, Holomastigotes magnum, Stephanonympha dogieli, Eutrichomastix termitis,* and *Devescovina elongata.* She also found *Microspironympha porteri* Koidzumi.

Tritrichomonas sp. (Duboscq and Grassé, 1929)

Host.—*Calotermes* sp. Lifou. Loyalty Islands.

Size 15–20 × 7–12μ. Three anterior flagella. Costa rather slender. Numerous eosinophile vacuoles in cytoplasm. "Resembles *Trichomonas* of insects and vertebrates." No figure given.

Besides this trichomonad, the only flagellate observed by Duboscq and Grassé in this termite was *Devescovina pruvoti.*

ORIGINAL OBSERVATIONS

Trichomonas termopsidis Cleveland, 1925

Plate 14, figures 6–11; pl. 15; pl. 16; figure B

INTRODUCTION

In addition to its previously recorded hosts, *Termopsis angusticollis* and *T. nevadensis, Trichomonas termopsidis* is present in *Termopsis laticeps* Banks from Arizona. In all its hosts the flagellates are abundant. In the following account a complete description is not attempted, but the study is limited chiefly to those points which supplement the work of Kofoid and Swezy (1919) and Andrews (1925).

Observations of living material were made both by transmitted light and dark-field illumination in .6 NaCl solution, in which *Trichomonas* continues active and apparently normal for some time. Vital dyes were used dissolved in absolute alcohol in 1 per cent solution, following the suggestion of Hall (1929). A film, limited in area according to the proper amount of dye, was placed on a slide and allowed to dry; the flagellates in a drop of salt solution were placed on this; and the edges were sealed with melted paraffin. *Trichomonas* is remarkably resistant to excessive concentrations of dyes which soon kill the other flagellates of *Termopsis.* Neutral red, Nile blue, Janus green, and brilliant cresyl blue were used.

The fixatives employed were Schaudinn's fluid with and without acetic acid, osmic vapor, Flemming's fluid without acetic, Champy's fluid, Zenker's fluid with and without acetic, Zenker-formol, 1 per cent chromic acid, concentrated $HgCl_2$, Bouin's fluid, and formalin vapor. Preparations were stained in Heidenhain's iron-alum haematoxylin, Kofoid's alcoholic iron haematoxylin (both mordant and stain very dilute, as recommended), Delafield's haematoxylin, Mallory's tricolor stain, and the Champy-Kull method. The best results for general structure were obtained by osmic vapor and Kofoid's alcoholic iron haematoxylin. For nuclear structure Schaudinn's fluid gives better fixation. The special values of the different reagents are considered in the treatment of various topics.

FORM AND ACTIVITY

The uncontracted form of the body is elongate, the costa and the undulating membrane following a broad spiral course and extending forward for a short distance after rounding the posterior end (pl. 16, fig. 21). In this form the anterior tip, where the flagella emerge, is narrowly pointed. The axostyle follows a more or less straight course, emerging at or close to the posterior end. In cross-section the body is circular or nearly so.

In the contracted state the body is stout and sub-orbicular; the costa has a C-form and lies in one plane; and the undulating membrane almost completely encircles the body (pl. 14, fig. 10; pl. 15, fig. 17). The body is markedly flattened.

Forward swimming is characteristic of the elongated form. The membrane undulates with such rapidity that when at maximum speed the eye cannot detect the separate curves. The characteristic movement of the contracted form is a constant circling in a very small radius, and sometimes the animal does no more than revolve in one position. The anterior flagella generally are united closely for about half their length, sometimes for the entire length. Distally the four flagella usually separate. The most proximal portion of the whip is held quite rigidly.

Active individuals are able vigorously to undergo changes of form, contracting into the C-form and revolving in a small circle once or twice; then extending the tip, straightening out and elongating the body, and swimming off in the same or another direction. An animal does not proceed far before it again contracts and revolves, to start off again, usually in another direction. This alteration of extension and forward swimming in a hasty, erratic way, and contraction and revolving, the undulating membrane "flowing" all the time, has been observed in many individuals in fresh preparations in salt solution. As the preparations become older more of the animals assume the contracted form and manner of movement.

SIZE

Kofoid and Swezy report a range in length from 16 to over 200μ, the giant individuals probably approaching multiple fission, and find that most individuals range from 75 to 125μ. Andrews found a range in 100 resting individuals from 11 to 55μ in length, and 6.8 to 28.8μ in width, averaging 29.17μ by 14.5μ. But in other colonies he encountered individuals 50 to 150μ long, most falling between 70 and 90μ. He suggested the possibility of racial or specific differences between these size groups.

Twelve hundred specimens were measured by F. M. Summers with the object of testing the possibility suggested by Andrews. He obtained a curve with a single maximum point at 34μ, and apart from minor fluctuations the curve was uniform. The average length of the twelve hundred specimens was 42.5μ, and the range from 9 to 85μ. In some hosts, many individuals are encountered in the prophase of mitosis, with two costas and duplicated flagella. He found these to average longer than the resting individuals, ranging from 30 to 85μ, and the largest number measured about 70μ. The length of the longest of the individuals with a single costa was 78μ. This work showed that there were no racial size differences in the *Trichomonas termopsidis* studied.

Since Summers did not encounter the giant individuals reported as reaching 150 to 200μ, and since the writer has not seen them, it is evident that the great size is unusual, even for dividing animals.

A test of the effect of starvation upon the size of *T. termopsidis,* was made by Summers. He starved healthy termites twelve days, until the hypermastigotes were all destroyed and the number of *Trichomonas* was greatly reduced. The length of the remaining individuals of *Trichomonas* ranged from 16 to 68μ.

It appears, then, that the size of *Trichomonas termopsidis* is similar to that of *Ditrichomonas termitis* as reported by Imms and Cutler, although the very small and very large individuals were not reported in that species.

GENERAL MORPHOLOGY

The clear area called the cytostome (pl. 14, figs. 6, 7) varies in breadth. In some it is only a narrow slit; in some it is broader in its posterior portion and almost closed anteriorly; in many it is, in section, as broad or broader than the nucleus in the middle, tapering at each end. It lies adjacent to the capitulum of the axostyle.

Covering the base of the group of four anterior flagella is a conical projection of the body. Along the ventral border of this is a deeply staining line, apparently continuous with or extending from the capitulum of the axostyle (fig. B, 1; pl. 14, figs. 10, 11).

The blepharoplast has been seen most clearly in some material fixed in osmic vapor and stained in Kofoid's alcoholic iron haematoxylin, in which it appeared as a large, solid, black granule (pl. 14, figs. 7–11; fig. B, 1). It lost its stain readily, and on the same slides in other individuals was pale, brown, or not visible at all. After Schaudinn's fluid it rarely stained with iron haematoxylin (pl. 14, fig. 6), though it sometimes stained faintly with Delafield's (fig. B, 2). In preparations made by the former method, consequently, it could easily be overlooked. In Mallory-stained material it took a deep blue color like the parabasal body and in contrast to the costa, which was red (pl. 16, figs. 21, 22). In Champy-Kull preparations the blepharoplast stained brown like the parabasal body, while the costa stained red. The rhizoplast (pl. 14, fig. 7), a single, straight filament from the blepharoplast to the nuclear membrane, has been seen clearly many times in Schaudinn-iron haematoxylin material, but not in other preparations.

The axostyle, parabasal body, and cytoplasmic inclusions have been studied in detail.

AXOSTYLE

The amount of projection of the axostyle varies as Andrews has pointed out, the projecting part occasionally equalling the body in length, but usually being only the tip of the axostyle. As the body contracts the axostyle is bent, so that its posterior end may be close to the blepharoplast, and is straightened out again when the body extends. The bending takes place chiefly in the region back of the nucleus (pl. 14, figs. 6, 10). The writer has not observed the extent of projection to change with change in the shape of the body.

Andrews states that the axostyle is "continually churning up the contents of the body." The writer has not observed any agitation of the cytoplasm by the axostyle; this structure appears to be moved passively as the body contracts and extends. In specimens whose activity is slowed down under the cover glass, so that the animal is quiet and the flagella and membrane move in a slower rhythm than normal, the projecting part of the axostyle sometimes is seen moving to and fro. In some cases, but not in all, these movements can be explained by contractions or movements of the body as a whole. In other cases, careful observation shows that they correspond to the rhythm of the membrane, and are caused by the undulations of the membrane striking against the flexible axostyle and causing it to bend slightly.

The axostyle is shown very distinctly in material stained in Kofoid's alcoholic iron haematoxylin after osmic vapor fixation, the sheath and endoaxostylar granules both staining deeply. The capitulum is broad, probably somewhat spoon-shaped, more convex on one side than the other, and lies on the left side of the nucleus. Anteriorly it ends at the centroblepharoplast, and from it a spur or filament extends along the conical cytoplasmic projection at the root of the anterior whip of flagella. Often it extends behind the nucleus. The capitulum may be observed especially distinctly in cases where the nucleus is out of position or is unusually small (pl. 14, figs. 8, 10, 11).

The trunk of the axostyle does not leave the extreme posterior end of the capitulum, but there is a sharp bend here and the trunk emerges at one side (pl. 14, figs. 6, 10, 11). As remarked by Andrews, the angle at which the trunk emerges is usually at least 90 degrees, and frequently the bend is greater than this. In elongated individuals, this bend is often less than 90 degrees, but most individuals in fixed material are more or less contracted. The portion just behind the post-capitular part is curved more or less sharply, sometimes very sharply, according to the degree of contraction of the animal. The posterior part of the trunk of the axostyle, amounting to about half of the trunk, is usually straight or slightly curved, the bending taking place chiefly in the more anterior section. This anterior section is somewhat flattened; the posterior part is cylindrical. In many cases the axostyle is not enlarged at the posterior end (pl. 16, figs. 19, 20). But in many other cases the posterior end is enlarged in a plumb bob form, tapering to a point and extending in a short filament (pl. 14, figs. 6, 10). The diameter of the trunk is about 1½ (1–2) μ; the end when enlarged often is 3–4μ in diameter; and the terminal filament is often as much as 3μ long.

Small endoaxostylar granules are abundant both in capitulum and trunk (pl. 14, figs. 10, 11). In material killed in osmic vapor, lightly stained in iron haematoxylin and counterstained in acid fuchsin these granules took a deep red color, which was absent from other structures of the body in the cases considered. They almost always stain lightly with Delafield's and deeply with iron-alum haematoxylin after certain fixatives. After use of Mallory's triple stain the sheath of the axostyle is blue, the granules red; after Champy-Kull's stain the sheath is brown and the granules red. In number they vary, sometimes being arranged in a single line down the middle, sometimes being more abundant and occupying more space in the axostyle.

When *Trichomonas termopsidis* is stained in Lugol's solution the axostyle stains brown. The rest of the body may remain clear or take a diffuse yellow or brown color. When the body is unstained, the axostyle often stands out very distinctly, stained brown or yellow brown from behind the nucleus to its posterior end. Often its whole substance is colored, but in some the endoaxostylar granules and sheath are stained more deeply than the rest. This staining reaction may be taken, as Alexeieff (1929) points out in the case of *Tritrichomonas augusta,* as indicative of the presence of glycogen or a similar substance in the endoaxostylar granules and the axostyle.

In many early division stages the anterior part of the axostyle had departed from its position near the nucleus, and the posterior end was in some cases excessively enlarged, due probably to degeneration (pl. 15, figs. 13, 14). In a late division stage, in which plasmotomy had been delayed and two new chromatic basal rods were growing in addition to the two large ones, the two new axostyles were well developed, though rather short (pl. 14, fig. 11). Each had an enlargement extended into a rather long filament at the posterior end, and contained the usual complement of endoaxostylar granules.

In *Ditrichomonas termitis,* Cutler (1919) somewhat hesitantly described division of the axostyle. This is very improbable, however; it seems likely that outgrowth is the general method of origin of new axostyles, the old axostyle being resorbed. This has been established in several other species of Trichomonadinae, as well as in Pyrsonymphidae (Kirby, 1924) and Oxymonadidae (Kirby, 1928; Zeliff, 1930; Connell, 1930).

THE PARABASAL BODY

The parabasal body is a long, narrow structure, ½ to 1μ in diameter and in larger flagellates frequently 30 to 45μ long. It is of even diameter from near the anterior end, where it tapers abruptly or is rounded off, to the posterior end, where it is generally rounded (fig. B, 2). It is somewhat but not very much flattened and is connected by a filament to the centroblepharoplast. Its anterior portion lies between the nucleus and the costa; its posterior portion is straight or variously twisted or coiled. Not infrequently it takes one, two or three loose turns around the axostyle, much as in *Metadevescovina debilis*. In a few instances it is rather tightly coiled back of the nucleus, as in *Devescovina*. In several specimens the parabasal body was split into two parts in its posterior portion, and in one case there was an extra limb anteriorly (pl. 16, fig. 22). Such cases are rare, and probably have nothing to do with reproduction of the structure, since origin by outgrowth has been demonstrated (p. 191).

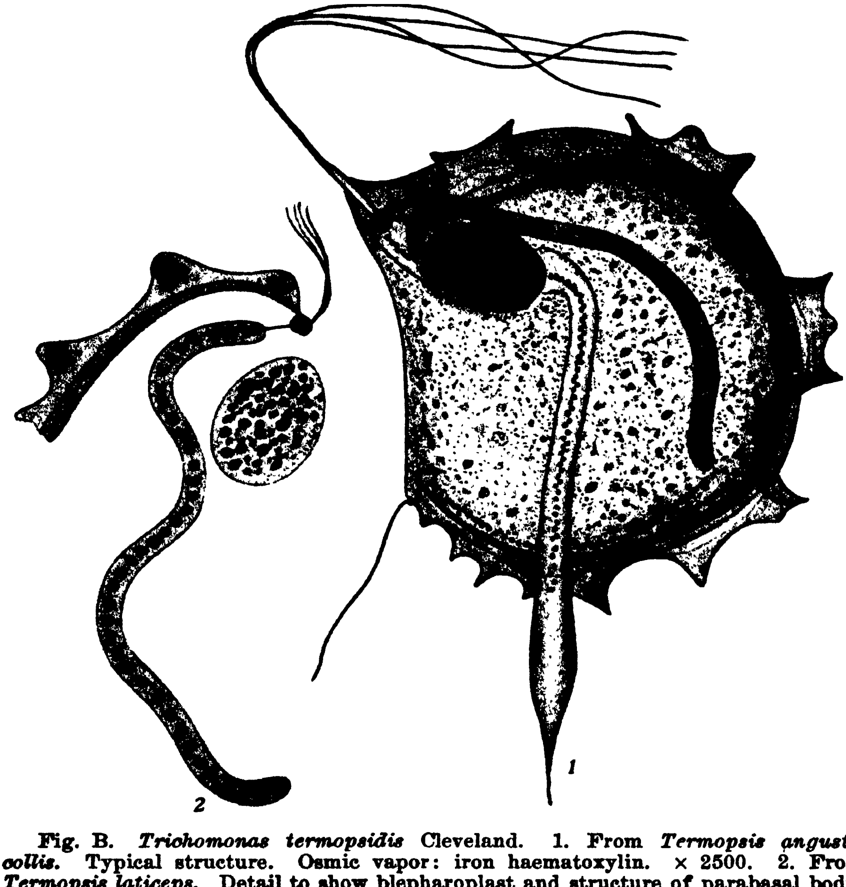

Fig. B. *Trichomonas termopsidis* Cleveland. 1. From *Termopsis angusticollis*. Typical structure. Osmic vapor: iron haematoxylin. × 2500. 2. From *Termopsis laticeps*. Detail to show blepharoplast and structure of parabasal body. Schaudinn's fluid: Delafield's haematoxylin. × 2560.

With iron-alum haematoxylin it has been possible to demonstrate the parabasal body after all the fixatives used except Schaudinn's fluid, concentrated $HgCl_2$ and formalin vapor. If acid fuchsin or erythrosin is used as a counterstain after Schaudinn's fluid and iron haematoxylin, the parabasal takes a reddish color and can be faintly traced (pl. 14, fig. 6), but it would not be recognized unless known from other material. It is most clearly shown after Champy's fluid, when it stains as deep a black as the costa, but loses the stain more readily than this. After 1 per cent chromic acid and osmic vapor it also appears clearly. In comparison with its distinctness after these fixatives, it shows rather obscurely after Flemming's without acetic. After fixation in the vapor of glacial acetic acid the parabasal body of *Trichomonas termopsidis* stains very poorly with iron-alum haematoxylin. In many cases, however, it is visible, and it appears to consist of a series of vacuoles in a darker matrix, as it often does after osmic vapor. After Zenker's followed by Mallory's triple stain the parabasal stains blue (pl. 16, figs. 21, 22), after Champy-Kull brown, while the costa is red in both cases.

In iron haematoxylin preparations following fixation in Champy's fluid, 1 per cent chromic acid, or osmic vapor the parabasal thread extending along one side of the body and connecting it to the blepharoplast is clearly visible (pl. 14, fig. 10). In some cases, when the substance of the body has almost lost its stain, the thread remains black. As pointed out by Andrews, the thread is on the margin of the parabasal and not in its center, as Cutler (1919) claimed in *Ditrichomonas termitis*. In the same preparations the less stainable substance sometimes appears to contain a row of small vacuoles, as Duboscq and Grassé (1927) describe in *Devescovina hilli* and other species; but in no case could this be seen clearly enough to leave no doubt as to the interpretation. There are certainly clearer areas in the body, but in these preparations the outlines of these and of the more stainable areas were too poorly defined for accurate analysis.

However, a very well defined internal structure has been seen in preparations stained in Delafield's haematoxylin, especially after use of Schaudinn's fluid with or without acetic acid, or concentrated $HgCl_2$. In these (fig. B, 2; pl. 15, figs. 12–16) the sheath of the parabasal body is well defined, but the thread is not conspicuous. Within the sheath is a row of relatively large granules which stain well but not as deeply as the chromatin of the nucleus. The granules are evenly spaced and located under one edge, probably the edge where the thread is located. In each of two parabasals 30μ long there were thirty-four granules, and one 46μ long contained forty. The substance surrounding the granules stains lightly or not at all.

Although most clearly seen in preparations made as above, this structure has been demonstrated by other technique. When Delafield's is used after formalin vapor the space occupied by the parabasal body appears perfectly clear, except that within it is the usual row of well stained granules. After Champy's fluid the parabasal stains fairly well with Delafield's and shows these internal granules. The same is true after 1 per cent chromic, Bouin's fluid (with acetic), and osmic vapor, in all of which the staining is fainter. In osmic vapor material (in which the fixation seems less perfect) the structure is least clearly recognizable. In iron-alum haematoxylin material this row of granules is generally unrecognizable, or the granules are unstained, but they have been seen clearly after Champy's fluid with Regaud's haematoxylin. It is evident from these results that the structure described above is a real structure independent of the fixative used.

A similar structure has been found in the parabasals of *Metadevescovina debilis* from *Kalotermes hubbardi* fixed in Schaudinn's fluid, *Devescovina* sp. from *K. minor* fixed in concentrated $HgCl_2$, and in *Devescovina striata* var. *hawaiensis* from *Neotermes connexus* fixed in Schaudinn's fluid. The parabasals of these flagellates stain more deeply with Delafield's than do those of *Trichomonas*, and often they appear of homogeneous composition. But when more critically destained, their appearance in *Metadevescovina* is that of striped bands, deeply stained and clear areas alternating. The chromatic blocks are larger than in *Trichomonas termopsidis*, and extend completely across the parabasal. In *Metadevescovina debilis* sometimes the clear areas are enclosed by the chromatic part. In *Devescovina* sp. from *K. minor* the parabasal contains granules similar to those of the parabasal of *Trichomonas termopsidis*.

REPRODUCTION OF PARABASAL BODY

Plate 14, figure 9; plate 15, figures 12–16

There has been considerable doubt as to the method of origin of new parabasal bodies in trichomonad flagellates. Andrews (1925) states that the parabasal body splits in *Trichomonas termopsidis*. Grassé (1926) believes that in *Trichomonas* the organelle undergoes longitudinal fission, and he figures this process in *Tritrichomonas batrachorum*. Cutler (1919) supposed division of the structure to take place in *Ditrichomonas termitis*, since he never observed intermediate stages of outgrowth. According to Duboscq and Grassé (1927) fission of the parabasal body occurs in *Devescovina hilli*, and a figure of this is shown (their text fig. H). Janicki (1915) believed that the structure divides in *Devescovina striata* var. *hawaiensis;* he was unable to follow this process, but later stages of division showed two well developed parabasal bodies of equal length. Wenrich (1921), studying *Tritrichomonas muris*, noted short parabasals in dividing flagellates, one shorter than the other, and suggests the possibility that one is the old one, perhaps with a portion detached, and the other is a new one growing out. In the Calonymphidae studied by Janicki (1915) the old parabasal is retained by one individual, and one new one is differentiated. Alexeieff (1924) states that during division of *Tritrichomonas augusta* the old parabasal is resorbed and two new parabasals are constituted at the expense of the plasmatic mitochondria. It has been possible to determine with certainty that outgrowth is the method of origin in *Trichomonas termopsidis:* the old parabasal is resorbed and two new ones are developed.

Redifferentiation of the parabasals begins in the prophase and is completed by the late anaphase before the two daughter nuclei have separated. In the earliest stages observed (pl. 15, figs. 12, 13) the nucleus is in a rather advanced prophase and the old parabasal is detached and lies in the cytoplasm behind the nucleus. The two new parabasals are short, slender, about 1½ times the length of the nucleus, and taper to points posteriorly. This material was fixed in Schaudinn's fluid and stained in Delafield's haematoxylin, and both the old and new parabasals contain the characteristic granules. In the new parabasals, especially in the tapering posterior portion, the granules are very small. In later stages (pl. 15, fig. 14) the new parabasals have increased equally in length, each tapering to a point posteriorly, and the old parabasal has diminished in size. By the late anaphase the old parabasal is disappearing, only a portion, with the granules coalesced and degenerating, remaining (pl. 15, fig. 15); and by the early telophase this has disappeared. In the final stage shown (pl. 15, fig. 16), with the nucleus in the late anaphase, the two new parabasals are of almost full length and their posterior ends are rounded, as in ordinary individuals.

A good many instances of this process have been seen in material from *Termopsis laticeps* prepared by the technique mentioned above. Some instances have been found in material from *Termopsis angusticollis* fixed in $HgCl_2$ and stained in Delafield's, and fixed in osmic vapor followed by iron haematoxylin (pl. 14, fig. 9).

As the parabasals are differentiated quickly, one may easily overlook the stages of outgrowth of the new and degeneration of the old structures, and, finding two well developed bodies in place of the one which was present in stages not much earlier, suppose that division of this structure has occurred.

CYTOPLASMIC INCLUSIONS

Plate 15, figures 17, 18; plate 16

In many individuals the cytoplasm is closely crowded with small fragments of wood, about the size of the smaller pieces ingested by *Trichonympha*. *Trichonympha* ingests many very small particles and also many larger ones; *Trichomonas* usually limits itself to the smaller pieces, but large pieces, which distort the body, are sometimes taken in. The majority of *Trichomonas termopsidis* from wood-fed hosts have so many of these fragments that the internal structure of the body cannot be seen, except that the nucleus stands out as a clear area in living material. The nucleus is closely surrounded on all except the ventral side by wood particles. Some individuals have less wood, others only a few small fragments, and a few have none at all. Andrews has pointed out the advantages of starving the hosts for a week to kill the hypermastigotes, and subsequently feeding them on filter paper, in preparation of material for study. The flagellates ingest some filter paper, but this does not interfere with cytological observation as does wood. The wood in the cytoplasm stains red or yellowish with neutral red (pl. 16, fig. 19), blue or green in Nile blue, purple in Janus green, and green in brilliant cresyl blue (pl. 16, fig. 20).

In those individuals free from abundant wood there are conspicuous many highly refractive granules which are generally spherical but often somewhat irregular in outline (pl. 15, figs. 17, 18). The number of these is variable, but usually they are numerous and in some cases the cytoplasm is full of them. They are most conspicuous when there is little wood, but they are present, though obscured, when the body is crowded with wood. In all they are of diverse sizes, ranging from minute up to 2 and occasionally 3μ or more in diameter. With neutral red some of these granules stain red (pl. 16, fig. 19) while others, often the greater number, do not stain at all. Similar differences in the stainability of the granules and spherules are obtained with Nile blue, where some take various shades of green and blue; and in cresyl blue, where some of the larger granules stain blue and then purple (pl. 16, fig. 20). Granules and spherules similar to these which stain with vital dyes are black in osmic vapor-iron haematoxylin preparations (pl. 14, figs. 10, 11). The animals have been placed in a hanging drop over 1 per cent osmic acid for eighteen days. During this period there was no blackening of any granules, although fat drops from the host were quickly blackened. In Zenker-Mallory material many of the larger granules stain red, some blue or purple (pl. 16, fig. 22).

In preparations made by the Champy-Kull method the cytoplasm of *Trichonympha* contains a large number of small, deeply staining red bodies in the form of granules or rods. They are present in about the same number and form in all individuals, and presumably they are the mitochondria of *Trichonympha*. In *Trichomonas* the few small granules grouped in a line or scattered under the costa stain red, but there are no structures easily recognizable as mitochondria like those in *Trichonympha*. The endoaxostylar granules and some of the larger spherules also stain red after the Champy-Kull technique.

Trichomonas barbouri sp. nov.

Plate 17; figure C, 1

Host.—Kalotermes (Glyptotermes) barbouri Snyder. Canal Zone.

The species of *Trichomonas* in *K.* (*Glyptotermes*) *barbouri* is fairly abundant, but far less so than is *Trichomonas termopsidis* in its hosts. It is associated with *Stephanonympha* sp., *Oxymonas barbouri* Zeliff, *Devescovina* sp., and *Tricercomitus divergens* Kirby. It has been named *Trichomonas barbouri* in honor of Dr. Thomas Barbour, for whom the host also is named. Dr. Barbour is in large part responsible for the splendid opportunities afforded biological workers by the Barro Colorado Island Biological Laboratory, where much of the material reported in this series of papers was obtained.

Extended individuals are rounded anteriorly and posteriorly, of about equal width along the longitudinal axis or somewhat tapering posteriorly, and the costa and undulating membrane are spiralled about 1½ turns, turning anteriorly for a short distance after rounding the posterior end. In partly contracted individuals the ventral side is often straight or concave, the anterior end broadly rounded, and the posterior portion usually tapering. Much contracted individuals are almost as broad as long, the costa forms an almost complete ring, and the projecting part of the axostyle is at the level of the nucleus. There is a conical extension of the body along the base of the group of anterior flagella (pl. 17, figs. 25, 27, 33) similar to that of *Trichomonas termopsidis.*

The average size of fifty individuals on two slides fixed in Schaudinn's fluid was 17.5 × 9μ, and the range in length from 11–24, in breadth 6–14μ. The largest individual encountered (pl. 17, fig. 33) was 23 × 17½μ. The four anterior flagella were 15–20μ long in most cases, and 25μ in this large individual.

The cytostome is a clear, crescentic area ventral to the nucleus. The undulating membrane is well developed, 1½ to 2μ high, and is thrown into folds similar to those of *Trichomonas termopsidis.* The costa is moderately developed to a degree comparable to that of *Tritrichomonas muris* and *Trichomonas trypanoides.* Apparently it is considerably stouter than that in *Trichomonas termitidis* and *T. macrostoma.* In one case, apparently degenerate, the substance of the costa was broken into a series of deeply staining blocks.

The blepharoplast, fairly large in size, is usually hemispherical in form, and very frequently the inner side is concave, though sometimes straight (pl. 17, figs. 24, 25). Its length is between ½ and 1μ. It is clearly demonstrable in Schaudinn-iron haematoxylin material, being much more readily stainable by this method than is the blepharoplast of *Trichomonas termopsidis.* When heavily stained it appears solid, but when it is more critically destained it may be clearly determined that it is composed of several small granules imbedded in a more lightly staining matrix (pl. 17, fig. 26). The four anterior flagella and the border of the undulating membrane emerge at the anterior end of the blepharoplast; the costa is attached by a short filament to the middle of the straight or concave side, and the parabasal thread is attached near the posterior end (pl. 17, fig. 24). A very delicate rhizoplast can be seen in a few cases extending from the blepharoplast to the nuclear membrane (pl. 17, figs. 24, 28, 33).

Close to the blepharoplast a structure, hitherto undescribed in *Trichomonas,* has been demonstrated clearly in Schaudinn-iron haematoxylin material, and less clearly in Flemming- and Bouin-fixed material. This the writer has called the parablepharoplast bar. It is a straight or slightly curved bar, situated ventral to the blepharoplast at the edge of the base of the conical cytoplasmic extension around the anterior flagella (pl. 17, figs. 24–26; fig. C, 1). There is some variation in its size; often it is about 1½μ long. Usually it is slightly curved inward at both ends. From the posterior end of the parablepharoplast bar a filament extends at an angle of about 135 degrees and passes to the nucleus. It extends around the nucleus on the right side against the membrane for a short distance, beyond which it cannot be traced because obscured by the heavily staining chromatin (pl. 17, fig. 25). The capitulum of the axostyle is on the left side of the nucleus. This filament is always very distinct if suitably stained, and is as thick as the sheath of the axostyle. A fine line sometimes appears to be present between the anterior end of the bar and the blepharoplast (pl. 17, fig. 24), but the existence of this is uncertain.

This structure was not recognized until after the animals had been studied for some time. At first it was believed that it was an adherent bacillus, or perhaps a deposition of stain. But after observing it frequently, the writer came to the conclusion that it is a constant structure, and then was able to find it and the filament in all suitably stained specimens.

The parabasal body is well developed (pl. 17, figs. 23, 24, 29, 33). It was best demonstrated in preparations stained with iron-alum haematoxylin after Flemming's fluid without acetic. After Schaudinn's and Bouin's fluids, though unstained, it can sometimes be seen. It does not stain with Delafield's after Schaudinn's fluid. In shape and relative size this body is similar to that of *Tritrichomonas muris* as described by Wenrich (1921). After its attachment to the blepharoplast it enlarges, soon reaching the maximum diameter which is maintained throughout its length. For a short distance it runs parallel to the costa; then it turns at a right angle and extends close to the nucleus on the dorsal side. It extends for a certain distance posterior to the nucleus, being once or twice the nuclear diameter in length. Its posterior end is usually rounded as in *Trichomonas termopsidis*, but occasionally it is pointed and sometimes is enlarged. Along the inner margin of the anterior portion is a deeply staining line, which probably is the parabasal thread.

The axostyle (pl. 17, figs. 23, 33; fig. C, 1) is fairly stout. At its posterior end there is always a lance head like enlargement, the distal portion of which usually, but not always, projects from the cytoplasm. It terminates in a short filament. Anterior to the enlargement the axostyle narrows to an unusual degree. Some distance back of the nucleus it begins to enlarge again and expands abruptly just behind the nucleus into a broad capitulum. The capitulum, which in relative size exceeds that of the other trichomonads described in this paper, is situated on the left side of the nucleus. The endoaxostylar granules are arranged in a line or scattered irregularly in all parts of the axostyle. The sheath stains well with iron haematoxylin.

The nucleus is broadly ellipsoidal or spheroidal. Its average size in twenty-four cases was 4 × 5μ, ranging from 3 × 3½ to 5½ × 6μ. When heavily stained it shows a well defined membrane, a large, dense chromatin mass with irregular outline, and lines extending across the narrow clear zone to the membrane (pl. 17, fig. 23). It stained very much more intensely than the nucleus of *Devescovina* on the same slides. The chromatin mass is resolved on destaining into a group of chromatin granules. In some nuclei the entire space within the membrane is filled with chromatin in granules or irregular, interconnected masses (pl. 17, figs. 24, 33). This is frequently in the form of a dense reticulum, extending throughout the intranuclear area save for a narrow space, or none at all, under the membrane.

The cytoplasm contains particles of wood and various granules.

Outgrowth of the new costa begins early, long before there are any other evidences of preparation for division (pl. 17, figs. 27, 30). The paradesmose (pl. 17, figs. 31, 32) is moderately stout and is slightly enlarged at the ends. The ends are connected by filaments to the blepharoplasts, which retain connection with the flagella and costas. This situation is comparable to that in *Trichomonas termopsidis*.

Trichomonas cartagoensis sp. nov.

Plate 18, figures 34–39; figure C, 2

Host.—*Kalotermes* (*K.*) *contracticornis* Snyder. Cartago, Costa Rica.

This flagellate was not very abundant in the hosts from which smears were made. It is associated with *Trichonympha* sp., a devescovinid similar to *Macrotrichomonas*, a smaller devescovinid similar to *Devescovina*, another undescribed trichomonad flagellate (not in Trichomonadinae), *Oxymonas* sp., and *Tricercomitus divergens*.

The shape of the body is much like that of *Trichomonas termopsidis* and *T. barbouri*. In the extended form the costa and undulating membrane take a long spiral course and the axostyle emerges near the posterior end; in the contracted condition these internal structures are curved in a C-form, the ends being close to the anterior end of the body. The average measurements of fifty fixed individuals were 19.8 × 10.5μ, with the range in length 14–29 and in width 6–14μ. The proportion of length to width was 3.7 :1 in a typical extended individual, and 1.2 :1 in a typical contracted individual.

The cytostome (fig. C, 2; pl. 18, figs. 34, 35) is similar in form to that of *Trichomonas termopsidis.* It is conspicuous as a broad, crescentic area bordered dorsally by the edge of the capitulum. There are four anterior flagella, 15–20μ long, and a conical extension of the body at the base of this group. The undulating membrane is fairly high, though much more delicate in appearance than is that of *T. termopsidis.* Sometimes a short free flagellum at the posterior end of this has been observed (pl. 18, fig. 36). The costa is moderately developed as in *Trichomonas barbouri.*

The blepharoplast is a granule of fair size, a little smaller than in the preceding species. A parablepharoplast bar like that of *T. barbouri* is present, but the material was not very favorable for study of this. The distance between the blepharoplast and the nucleus is variable, but usually not more than a quarter or a third of the length of the nucleus. A rhizoplast was not seen.

The axostyle (pl. 18, fig. 34; fig. C, 2) is similar to that of *Trichomonas termopsidis.* There is a broad capitulum, at one side of the posterior portion of which the trunk of the axostyle leaves. The trunk is curved rather sharply beyond the point of origin. In the capitulum and the trunk are a few scattered granules, but they were inconspicuous in this material. The posterior end of the axostyle is enlarged slightly, though not as much as in the other species, before tapering to a point and terminating as a short filament. It often projects from the body for a short distance. One or two small periaxostylar rings (pl. 18, fig. 36) have been observed in some instances.

The parabasal body (pl. 18, figs. 34, 36; fig. C, 2), which was fairly conspicuous in some material stained in Delafield's after Schaudinn's fluid, is slender and very short. In this material it invariably appeared as a row of chromatic blocks or granules, curved on a short radius, and often hardly passing the level of the anterior end of the nucleus. In preparations stained in light green after alum carmine, the parabasal stained a deeper green than the surrounding cytoplasm and appeared homogeneous.

The nucleus is spherical or broadly ellipsoidal, measuring about 3×4 to $4 \times 5\mu$. The interior is almost completely filled with a mass of chromatin. In some the visible structures are irregular blocks of chromatic material together with numerous smaller granules. Often there is a large, irregular, deeply staining mass around which are smaller granules (pl. 18, fig. 36; fig. C, 2). Usually there is a clear space of small extent beneath the nuclear membrane, but in some cases the interior is completely filled with the chromatic material.

The paradesmose (pl. 18, figs. 37–39) is moderately stout, and its ends are connected by filaments to the blepharoplasts. The chromosomes are short, rod-like bodies, numbering about six.

The cytoplasm of *Trichomonas cartagoensis* contains particles of wood and other ingested objects.

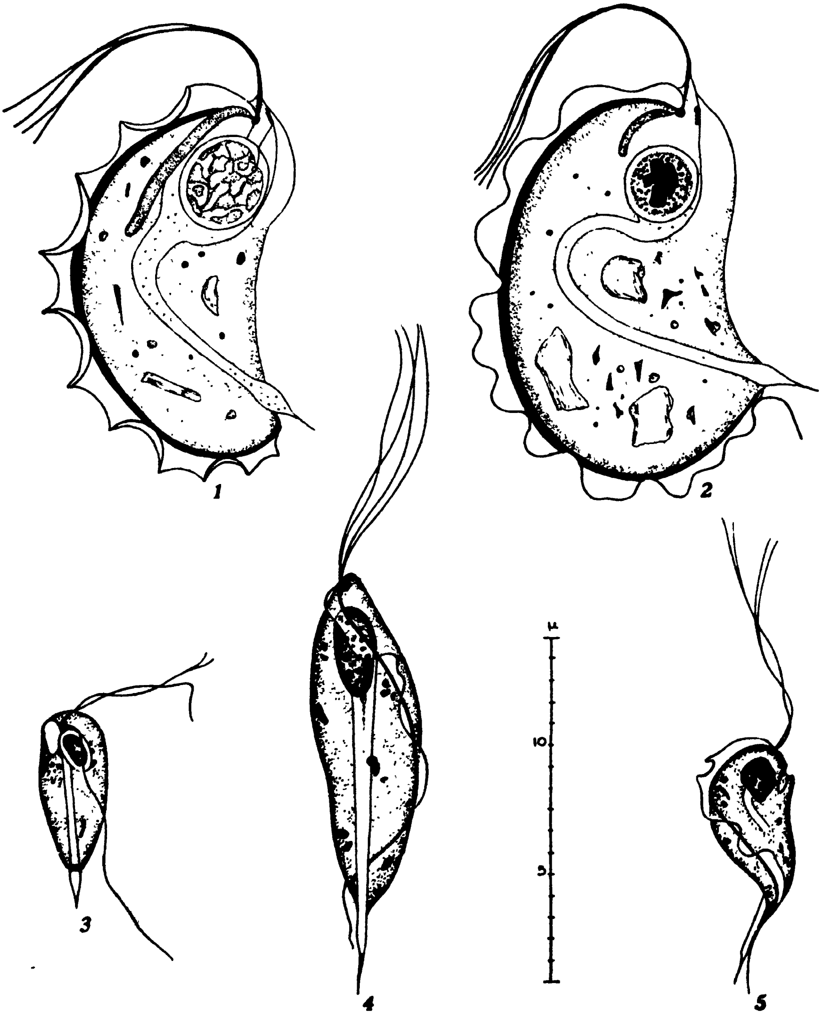

Fig. C. 1. *Trichomonas barbouri* sp. nov. from *Kalotermes* (*Glyptotermes*) *barbouri.* 2. *Trichomonas cartagoensis* sp. nov. from *K.* (*Kalotermes*) *contracticornis.* 3. *Eutrichomastix axostylis* sp. nov. from *Nasutitermes* (*Subulitermes*) *kirbyi.* 4. *Trichomonas linearis* sp. nov. from *Orthognathotermes wheeleri.* 5 *Trichomonas labelli* sp. nov. from *Mirotermes hispaniolae.* All × 3000.

Trichomonas linearis sp. nov.

Plate 18, figures 40–45; figure C, 4

Host.—Orthognathotermes wheeleri Snyder. Canal Zone.

This small flagellate is the only protozoan which has been found in the intestine of *Orthognathotermes wheeleri,* where it is fairly numerous.

A conspicuous characteristic of the animal, which has led to selection of the name *linearis,* is its straightness. The axostyle is always straight or only slightly curved, and with it the body maintains a linear form, more or less spindle-shaped, often tapering more at the posterior end. It is circular in cross-section. The axostyle projects posteriorly for a distance usually not exceeding 6μ without the terminal filament, but occasionally equalling the rest of the body (pl. 18, figs. 41, 42). Fifty individuals ranged in length from 9 to 24μ, in width from 3 to 8μ. Twenty-five specimens, measured to the end of the body not including the projecting part of the axostyle, averaged 15μ; twenty-five others, measured to the end of the axostyle without the terminal filament, averaged 17.4μ. The average breadth of these fifty flagellates was 4.25μ.

The cytostome (pl. 18, fig. 44) is present but inconspicuous. There are four anterior flagella about 15μ long. The conical extension of the cytoplasm at the base of the group of flagella, seen in the three previously described species, was not observed in this flagellate. The flagella emerge from the cytoplasm almost immediately after their origin from the blepharoplast. The undulating membrane is not as high as in the preceding species. It, with the costa, always follows a long, leiotropic spiral course. The undulating membrane extends into a free posterior flagellum several (often 3–5) microns long. The costa is very slender, and in its posterior portion is usually an inconspicuous filament not much stouter than a flagellum.

The large blepharoplast is located at the anterior tip of the body; there is no considerable amount of cytoplasm anterior to it. It is unevenly spherical and when sufficiently destained appears to be made up of several (3–5?) granules imbedded in a common matrix (pl. 18, fig. 43). A rhizoplast was not seen despite careful search for this structure. The material was not favorable for determination of the parabasal body. It was not found in Schaudinn-Delafield preparations.

The axostyle is somewhat broader than elsewhere in its anterior portion, but it is not expanded into a prominent capitulum as in the preceding three species. It follows a straight or slightly curved course to emerge from the body at the extreme posterior end. Back of the nucleus it is of approximately even diameter, varying from less than a half to almost a micron in different individuals, until it tapers to a point without any enlargement. It terminates in a short filament, which sometimes stains deeply while the remainder of the structure is hyaline. An indistinct line of minute granules is sometimes visible extending longitudinally through the axostyle. This is conspicuous just back of the nucleus, where it broadens.

The nucleus is usually elongate ellipsoidal, ranging from 1 × 2½ to 3 × 6, averaging 1.5 × 4μ. In a few cases, however, it is almost spherical. It is generally situated at a very short distance, two microns or less, from the blepharoplast. The space within the membrane is completely filled with small chromatin granules, which are more densely packed in some nuclei than in others.

Numerous relatively large stainable granules are scattered in the cytoplasm (pl. 18, fig. 43). No fragments of wood or other ingested solid material were observed.

The paradesmose (pl. 18, fig. 45) is slender, hardly more than twice the thickness of the group of two flagella attached to each blepharoplast. The chromosomes are short, rod-like bodies probably numbering five or six.

Trichomonas labelli sp. nov.

Plate 19, figures 46–49; figure C, 5

Host.—*Mirotermes hispaniolae* Banks. Canal Zone.

This flagellate occurs in very small numbers in its host, where it is associated with an abundant fauna of amoebae. These amoebae have been described (Kirby, 1927) as *Endamoeba disparata, Endamoeba majestas,* and *Endolimax termitis.* This completes the list of Protozoa in *Mirotermes hispaniolae.* No flagellates were found in *Mirotermes panamaensis.*

The organism appears in the usual elongate form, tapering posteriorly, with the undulating membrane slightly turned in a leiotropic spiral; or it is contracted and more rounded. In twenty-five individuals the length ranged from 6–11 and the breadth from 3–7μ, averaging 8.4 × 4.7μ. A structural features of the organism is an occasional lip-like projection in the region of the cytostome (pl. 19, fig. 47; fig. C, 5), though this is to be found only in a small proportion of individuals. It is similar to the lip described in the larger *Trichomonas macrostoma* by Dogiel (1916).

There are four anterior flagella, about 15μ long, which adhere to one another for a short distance beyond the blepharoplast. The undulating membrane is of fair size and at its posterior end there is a rather long free flagellum (pl. 19, figs. 48, 49). The costa is rather slender, but is relatively stouter than is that of *Trichomonas linearis.*

The moderately large blepharoplast is situated at the tip of the body. As in *T. linearis,* there is very little cytoplasm between it and the anterior membrane. In some cases it is irregular in outline, as if composed of a group of granules. No parabasal body has been found in this material.

The hyaline axostyle (pl. 19, fig. 49) is similar in form to that of *Trichomonas linearis,* but it is relatively more slender. From back of the nucleus, where it is slightly expanded, it extends through the body and usually projects for a distance of 2 to 7μ at the posterior end or on the ventral side. At its end the axostyle tapers without previous enlargement and terminates in a short filament.

The nucleus is spheroidal or broadly ellipsoidal, placed at a distance of half its diameter or less from the blepharoplast, and averages about 1½ × 2μ in size. The range in twenty cases was from 1 × 1 to 2 × 3μ. The space within the nuclear membrane is entirely filled with chromatin granules, fewer in number and relatively larger than in *T. linearis* (pl. 19, fig. 47). In some very much destained material a large granule (karyosome) in a clear space appears when the rest of the granules are not visible (pl. 19, fig. 48).

Tritrichomonas brevicollis sp. nov.

Plate 19, figures 50–58; figure D, 2

Host.—*Kalotermes* (*Calcaritermes*) *brevicollis* Banks. Canal Zone.

This flagellate occurs in small numbers in its host, where it is associated with *Calonympha* sp., *Gigantomonas* sp., *Devescovina* sp., *Oxymonas ovata* Zeliff and a large, multinucleate oxymonad named *Kirbyella zeteki* by Zeliff (1930). No trichomonad of this subfamily, except *Tricercomitus,* has been found in *K.* (*Calcaritermes*) *emarginicollis.*

In all of the material examined, which was fixed, the undulating membrane is curved around the rounded posterior end, and the axostyle projects near or a little back of the middle of the ventral side.

The anterior end is drawn out into a beak-like process, covering the base of the anterior group of flagella. The average size is about 13.2 × 6.6μ, the range in length 10–17 and in width 4–8μ. The cytostome (pl. 19, fig. 50; fig. D, 2) is a long, subtriangular, clear area beginning near the blepharoplast and extending over to the middle of the left side of the body, where it is pointed. In one case it was 8μ long.

There are three unusually long anterior flagella of 20–25μ. The undulating membrane is well developed and is thrown into prominent folds. The costa is rather slender, a little more so than in *Trichomonas cartagoensis*. In the material studied, it rounds the posterior end and extends forward for a quarter to a half or more of the body length, where its attenuated end passes outward along the projection where the axostyle emerges from the body (pl. 19, figs. 50, 52). In a few instances in which it was measured, the costa was 20–25μ long.

The blepharoplast is a granule of fair size located at the base of the conical anterior tip and from it a rhizoplast extends to the nuclear membrane (pl. 19, fig. 55). The parabasal body (pl. 19, fig. 56), which has been seen clearly in material fixed in Flemming's without acetic and stained in iron haematoxylin, is a short slender rod. Its posterior portion lies close to the nuclear membrane, and it does not extend back of the posterior end of the nucleus.

The axostyle in all cases projects from the body for a short distance at a point varying from two-thirds of the distance back to the level of the nucleus. It is fairly stout, tapers to a point without any marked enlargement, and terminates in a short filament.

The nucleus is spherical or broadly ellipsoidal and about 3μ in diameter. Its distance from the blepharoplast varies from very close to about half the nuclear diameter. It is surrounded by a distinct membrane and contains a large chromatin mass, about 2μ in diameter, separated by a fairly extensive clear space from the membrane (pl. 19, figs. 52, 55; fig. D, 2). The clear zone often appears to be traversed by spoke radii. When suitably destained the chromatin mass shows a granular composition. As this nuclear structure is the principal point of distinction between this and the following species, special care was exercised in determining that it is constant.

The cytoplasm often contains fragments of wood, large spherules in vacuoles, and numerous granules of varying size, but most specimens contain very little ingested material. The flagellate is evidently xylophagous, at least in part.

The paradesmose (pl. 19, figs. 57, 58) is a stout bar, enlarged at the ends which are in contact with the nuclear membrane. These ends are connected by short filaments to the blepharoplasts (pl. 19, fig. 54). In the anaphase shown on plate 19, figure 57, the old axostyle is dislodged from its original position and two short axostyles extend from the blepharoplasts, crossing back of the nuclear spindle. In this stage, two flagella are attached to one blepharoplast and one to the other blepharoplast.

Tritrichomonas holmgreni sp. nov.

Plate 20, figures 59–64; figure D, 3

Host.—Kalotermes (*Neotermes*) *holmgreni* Banks. Panama.

This trichomonad is fairly abundant in *K.* (*Neotermes*) *holmgreni*, where it is associated with species of *Stephanonympha, Devescovina, Oxymonas,* and a small devescovinid, besides *Tricercomitus divergens.* It has been studied in living material from termites sent from Panama and kept in the laboratory at Harvard University for nearly two years by Dr. L. R. Cleveland, as well as from smears prepared at the Barro Colorado Biological Station.

In the majority of individuals in both living material and smears, the body form is that of *Tritrichomonas brevicollis.* Some individuals are more elongated, with the undulating membrane in a leiotropic spiral. Twenty-five fixed specimens ranged in length from 10–20μ, averaging 16μ, in width from 4–7μ, averaging 5μ, but from living material some were recorded as much as 40μ long. The cytostome is like that of *T. brevicollis.*

There are three anterior flagella about 20μ long. The undulating membrane is of moderate height, decreasing posteriorly. No free flagellum has been seen at the end of the membrane, even under darkfield illumination. The costa and blepharoplast are like those of the preceding species, and from the blepharoplast a rhizoplast extends to the nuclear membrane. The parabasal body (pl. 20, fig. 59, 60, 63; fig. D, 3) also is a short, slender, curved. clavate or rod-like structure which does not extend back as far as the posterior end of the nucleus. It is similar to that of *Tritrichomonas brevicollis;* if anything, it is smaller than this.

The axostyle (fig. D, 3) is similar to that of the preceding species, but not quite as stout. It tapers at the end without previous enlargement and terminates in a short filament. In most cases, especially in living material, the axostyle does not project from the body, but in some it does project for a short distance.

The nucleus is broadly ellipsoidal or spheroidal, measuring about $2 \times 3\mu$. It differs markedly in structure from that of *Tritrichomonas brevicollis,* appearing larger, though not actually so, due to the fact that it is richer in chromatin. The space within the membrane is entirely filled with numerous chromatin granules (pl. 20, figs. 59, 62), in contrast with the condition in the other species where the chromatin is concentrated in a central mass, leaving an extensive clear space under the membrane. The granules in the nucleus of *T. holmgreni* are separated enough so that even when nuclei are quite heavily stained the individual granules are discernible; while in the other species the same stain would show only a dense mass. It may be worth while remarking that these nuclei have been studied under comparable conditions and in a sufficiently large number of cases so as to avoid any danger of misinterpretation.

The cytoplasm contains numerous fragments of wood and many granules of diverse sizes.

Pentatrichomonoides scroa gen. nov., sp. nov.

Plate 20, figures 65–70; plate 21; figure D, 1

Hosts.—Kalotermes (Cryptotermes) dudleyi Banks. Canal Zone. *K. (Lobitermes) longicollis* Banks. Taboga Island, Panama.

Trichomonad flagellates with five anterior flagella are abundant in the two termites named above. In general organization and in the number of anterior flagella these resemble members of the genus *Pentatrichomonas,* but because of special characteristics they have been assigned to a new genus, *Pentatrichomonoides.* This organism has been studied alive and in preparations fixed in Schaudinn's fluid, Flemming's without acetic, or osmic vapor and stained in Heidenhain's or Delafield's haematoxylin. In some acid fuchsin was used as a counterstain. Most of the preparations from *K. (Cryptotermes) dudleyi* were made from termites brought alive from Panama by the writer. Thanks are due to Mr. J. Zetek for supplying some of this. Some studies were made of material brought from the Canal Zone by Mr. O. L. Williams. The smears of *K. (Lobitermes) longicollis* were made at Barro Colorado Island.

It was astonishing to find the same species of this peculiar trichomonad in these two hosts, for their other flagellates are for the most part quite different. In *K.* (*Cryptotermes*) *dudleyi* the multinucleate *Proboscidiella kofoidi* Kirby, 1928, which represents an advanced development in the *Oxymonadidae,* is present, while in *K.* (*Lobitermes*) *longicollis* that polymastigote family is represented only by *Oxymonas.* On the other hand, *K.* (*Lobitermes*) *longicollis* is distinctive in the possession of *Snyderella tabogae* Kirby, 1929, which is foremost among the

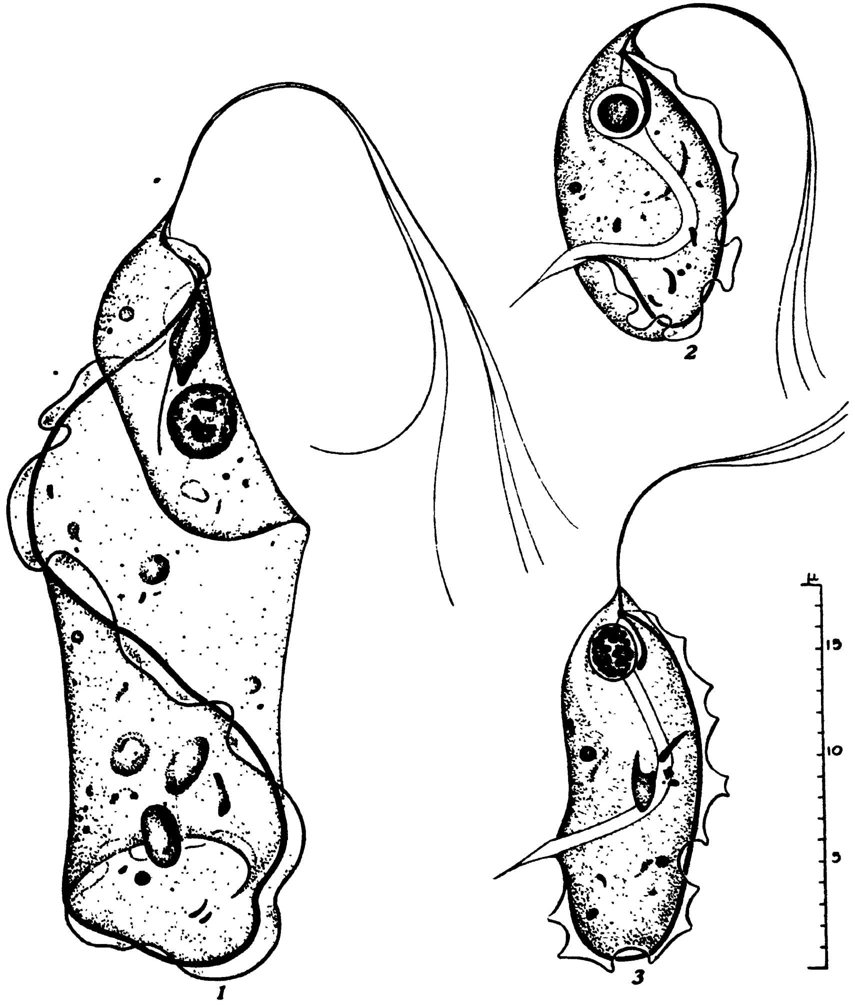

Fig. D. 1. *Pentatrichomonoides scroa* gen. nov., sp. nov. from *Kalotermes* (*Cryptotermes*) *dudleyi* and *K.* (*Lobitermes*) *longicollis.* 2. *Tritrichomonas brevicollis* sp. nov. from *K.* (*Calcaritermes*) *brevicollis.* 3. *Tritrichomonas holmgreni* sp. nov. from *K.* (*Neotermes*) *holmgreni.* All × 3000.

known species of Calonymphidae. *Hexamastix claviger* is present in *K. dudleyi* but not in *K. longicollis*. *Stephanonympha* and *Devescovina* are represented in both hosts, and *Tricercomitus divergens*, which is widespread in the Kalotermitinae, is present in both.

On the assumption that the protozoan faunas of termites are specific, and that cross-infections have not taken place, we have hoped to be able to correlate the phylogeny of the Protozoa (that is, of the flagellates) with that of their hosts. For the most part, distribution of the entozoic faunas is such that it seems possible for us to do this, but some facts present difficulties. Unless we admit a relatively recent cross-infection, we must suppose either that *Pentatrichomonoides scroa* has remained unchanged while the remarkable divergence of the remaining faunas of its hosts took place, or that here is a case of convergent evolution. With an ordinary or more generalized trichomonad there would be less difficulty with the latter two of these assumptions. *Tricercomitus* is present in many hosts and shows little differentiation. But *P. scroa* is an unusual form unlike any other trichomonad known.

The body form is unlike that of most other trichomonads of this subfamily, i.e., more or less flattened with the undulating membrane passing around the dorsal side or in a long spiral to the posterior end, or curved around this to continue for a greater or lesser distance up the other side. Rather it is more or less circular in cross-section, often squarely or obliquely truncate at the posterior end, acuminate at the anterior end, and, as a whole, conspicuously spiraled (fig. D, 1). From the anterior to the posterior end the undulating membrane and costa are wound in a leiotropic spiral of about one turn. These usually, but not always, continue along the truncate posterior border, then forward in a steeper curve for a short distance, and finally almost transversely nearly to the point where the membrane previously passed. There the costa is sharply incurved in a hook-like end. On the right side of the undulating membrane the body is, in many cases, more or less deeply grooved (pl. 20, figs. 66, 67; pl. 21, fig. 71). When this grooving is deep and broad, the anterior part of the body on one side is set off as a semilunar or crescentic area, straight or concave at its outer border. At the posterior end of this area the body is sometimes extended outward in an angle (pl. 20, fig. 66), so that, with the obliquely truncate posterior end, it has a rhomboidal outline. The scroll-like form of many individuals suggested the name *scroa* for this species. The scroll form, while characteristic, is not universal. Many individuals, especially in smears, are rounded out and do not show the grooving (pl. 20, fig. 65).

The range in size of 150 fixed individuals on six slides from *K.* (*Cryptotermes*) *dudleyi* was in length 18–45, in breadth 6–15μ. The average of these is 29.4 × 9.3μ. Fifty of them, on one slide, were smaller than usual, averaging 26 × 7.3μ, while the other hundred, from five slides, averaged 31 × 10.4μ. It appears to be true, at least in the case of several species of termites, that the Protozoa in certain hosts may be smaller in size than in others. Consequently not a great deal of significance can be attached to size variations of a few microns in different series of measurements.

The five anterior flagella are about 25 (18–30) μ long. That the number is five is certain; it has been counted repeatedly in living and stained specimens. In living material they are ordinarily united into a bundle, which is sometimes as compact as that of *Hexamastix claviger,* but often they are separated at the distal end. The flagella are separated for most of their length in fixed material. Around the base of the anterior whip is a small, conical extension of the cytoplasm (pl. 20, fig. 66). The undulating membrane is moderately shallow in proportion to the size of the animal. No free flagellum was observed at its posterior end under dark-field illumination, which would show it if present. The costa is slender and quite delicate toward the posterior end, where it is hardly stouter than the marginal flagellum. In the living animal the whip of anterior flagella beats constantly backward and is not advanced to its full extent before the animal. Meanwhile the membrane undulates rapidly and the body rotates. It is one of the most active flagellates in its hosts, and it survives longer than the larger ones in preparations in salt solution. No cytostome has been observed.

The moderate sized blepharoplast (pl. 20, figs. 69, 70; pl. 21, figs. 81, 82) is compact, elongated, crescentic, and lies transversely or obliquely with its concave side forward. From its anterior end arise the anterior flagella and the marginal flagellum of the undulating membrane. To its posterior end are attached the costa, the parabasal body, and the innominate filament described below (pl. 21, fig. 81). The writer has been unable to find a rhizoplast between the blepharoplast and the nuclear membrane.

The parabasal body lies between the blepharoplast and the nucleus, which is situated at about a quarter of the body length back of the blepharoplast. The form of the parabasal is unusual. From one aspect the main body is elliptical, pyriform, or almost circular, and is connected to the blepharoplast by a slender peduncle (pl. 20, figs. 65–69).

Sometimes it is broadly rounded at the posterior end, but frequently it tapers more or less. From another aspect the parabasal appears flattened on one side, where there is a more deeply staining line which probably is the parabasal thread (pl. 20, fig. 70). In the middle of the stoutest part there often appears a large, more deeply stained granule surrounded by a clear area (pl. 21, figs. 81, 82). This parabasal stains very faintly with Delafield's after Schaudinn's fluid, in contrast to the condition in *Devescovina, Stephanonympha* and *Snyderella* in the same preparations. It is well stained with iron-alum haematoxylin after fixation in osmic vapor or Flemming's without acetic, especially the former.

To the blepharoplast is connected a filament (pl. 20, fig. 66; pl. 21, figs. 80–82; fig. D, 1) which passes close to the surface of the body to end near or a short distance beyond the posterior end of the nucleus. This has been called the innominate filament. It usually runs close to the costa for a short distance, then turns off from it abruptly. At the level of the nucleus the costa and this filament lie almost on opposite sides of the body. Often the filament is straight, but frequently it is wavy, especially in its anterior portion. For the most part, its length is relatively constant.

At first it seemed possible that this filament was a parabasal thread, for often it lies over the parabasal body which is situated near the surface in the same region (pl. 20, fig. 66). Many instances, however, have been observed in which the filament is quite free from apparent contact with the parabasal body (pl. 21, fig. 82). Then it seemed possible that this is a new costa in an early stage of outgrowth. The fact that it is always of approximately constant length and is always present, strongly opposed this interpretation. The possibility was finally disposed of by the observation of several cases in which this filament is present in addition to two costas (pl. 21, figs. 80, 82). There is no doubt, therefore, that the innominate filament is a separate structure.

The only structure comparable to this which a survey of the literature on *Trichomonas* has disclosed is the filament in *Trichomonas vaginalis* which Hegner (1925) thinks is a cytostomal fibril, although no well defined cytostomal fibril has been described in connection with the cytostomes of other trichomonads.

There is a very delicate axostyle (fig. D, 1) which runs through the cytoplasm but does not project from the posterior end. This was not observed in stained preparations, despite careful search, nor could it be seen in living animals under transmitted light. Under dark-field illumination it could be seen faintly, showing two edges close together. It would be very easy to overlook this structure, the slight development of which is in marked contrast to the condition in other described Trichomonadinae from termites, except *Tricercomitus.*

The nucleus is usually located at about one quarter of the body length from the anterior end, and lies just beneath the surface at a point almost exactly opposite the costa at that level. In shape it is spherical or subspheroidal with some irregularity of outline. One surface or another is sometimes more or less flattened. Its diameter ranges from 2 to 5μ, but most frequently is about 3μ.

In the structure of the nuclei there is some variability with respect to the number, size, and distribution of the chromatin granules and other intranuclear structures. In the interior of the nucleus, usually excentrically placed, there is often a rather large granule (pl. 20, fig. 65; pl. 21, figs. 71–73). This granule stains more deeply than the chromatin with iron-alum haematoxylin, but with Delafield's haematoxylin it does not stain as well as the chromatin. Near it there is a larger granule or irregular mass which stains more faintly than the chromatin with Delafield's and iron haematoxylin (pl. 21, figs. 72, 73). These bodies are surrounded by a clear area, which is traversed by spoke radii. The chromatin granules are of various sizes, sometimes limited to the peripheral layer, sometimes distributed throughout the outer portion of the nucleus. In some smaller nuclei the chromatin granules are few, relatively large, and are placed in a single layer beneath the membrane (pl. 21, fig. 76). Usually there is no space between the outermost chromatin and the membrane, but in some cases (pl. 21, figs. 78, 79) there is a space between it and a chromatin mass on the surface of which chromatic granules are located. Possibly this last condition is due to shrinkage, but comparison with other material makes that seem unlikely.

The cytoplasm has rarely been observed to contain particles of wood, but it usually contains numerous vacuoles with solid contents (pl. 20, figs. 69, 70; pl. 21, fig. 85). These contents consist of granules, straight or curved rods of various sizes, and often larger spherical or ellipsoidal bodies. The animal appears to be xylophagous to a small extent if at all, feeding on bacteria, spores, and other organic material.

The paradesmose (pl. 21, figs. 83, 84) is band-formed and remarkably broad for a trichomonad. It does not stain as deeply with iron-alum haematoxylin as does the paradesmose in *Trichomonas termop-*

sidis and the other species described in this paper. In the telophase (pl. 21, fig. 83) which was the earliest division stage observed, its ends lie in depressions in the nucleus. It appears to connect the blepharoplasts, instead of being attached at the ends to the blepharoplasts by filaments as in *Trichomonas termopsidis, T. barbouri, T. cartagoensis,* and *Tritrichomonas brevicollis.* However, it may be that the filaments are very short. The anterior flagella are at this time distributed three and two between the two blepharoplasts.

Pseudotrypanosoma giganteum Grassi, 1917

Plate 22; compare figure A, 1

Hosts.—Porotermes adamsoni (Froggatt). Victoria, Australia. *Porotermes grandis* Holmgren. Victoria, Australia.

Associated with this trichomonad in *Porotermes adamsoni,* Grassi described *Joenina pulchella, Spirotrichonympha mirabilis,* and *Spirotrichonymphella pudibunda.* In *Porotermes grandis* hypermastigotes have been found very similar to, and apparently identical with these. According to G. F. Hill (1926) there are no structural differences between the two species of termites except that of size. The distribution is different, but continuous. "All the authenticated Victorian series of *P. adamsoni* are from low, sandy, coastal localities, whilst all the series here definitely referred to *P. grandis* are from heavily timbered hilly or mountainous districts at elevations of from about 800 feet to 3000 feet above sea level. The series of doubtful identity are those from the undulating country between the coast and the ranges and from the lower foothills and adjacent gullies" (Hill, 1926, p. 149). From the foothill country, Hill records material "which, being of uniform moderate size, might be attributed to either species in the absence of a more reliable character, which I have failed to discover."

The writer is indebted to Mr. Hill for material from *Porotermes grandis* collected in 1926 from Fern Tree Gulley, Victoria, Australia.

In form the large flagellates are often relatively slender, with the undulating membrane twisted in a spiral along a more or less linear body, or with the membrane along the dorsal edge of a curved body. Often the posterior portion is curved forward against the more anterior portion, so that the body is shorter and stouter. The large, slender individuals in the writer's material were from 145 to 205μ long and 20 to 40μ broad. These measurements are considerably greater than those calculated from Grassi's figures, but since Grassi gives no measurements in the text and these were derived from the reported magnifications, the true size of his forms is uncertain. The length was taken along the longitudinal axis of the body, whatever the curvature. In addition to these large forms, there have been found many small ones, more like typical trichomonads, 25 to 42μ long and 8 to 13μ broad. It has not been possible to determine with certainty whether or not these belong to the same species, for transitional stages were not found in the rather limited material at the writer's disposal. The larger forms will be described first, followed by an account of the smaller trichomonad.

There are three anterior flagella, not more than 30μ long, which is relatively short for an animal of 150 to 200μ. Grassi observed only two anterior flagella, but he found it difficult to distinguish them and may have overlooked the third. The undulating membrane is relatively low and is thrown into a great many shallow folds. The costa is very stout, often being broader than the membrane. From the point where it leaves the blepharoplast the costa broadens gradually to a width of 4 or 5μ, maintains this size for a short distance, then tapers to a slender posterior end.

The blepharoplast (pl. 22, figs. 86, 92) is a rather large granule, flattened or concave on the side where the chromatic basal rod joins it in the middle. This blepharoplast does not correspond to the several small granules described by Grassi at the base of the flagella, rib and membrane. In some iron-alum haematoxylin material the single large blepharoplast is not visible (pl. 22, fig. 87). It has been seen best in Delafield stained preparations. Grassi apparently did not observe the single large blepharoplast.

In the cytoplasm of the rounded tip of the body just anterior to the blepharoplast there is a convex, plate-like structure (pl. 22, figs. 86, 92) which is apparently connected to one edge of the blepharoplast. It appears as a curved filament, often deeply stained, in optical section, but it is visible at different optical levels. This structure evidently corresponds to the little tongue ("beccuccio o linguetta") described and figured by Grassi.

The nucleus is situated at a certain distance, about 20 to 25μ, from the anterior end. It is much elongated and usually tapering at the ends, where it is rounded or pointed. In a number of the large specimens the length ranged from 13 to 20μ, the width at the middle from 3 to 6μ. Beneath the membrane, which is very distinct, is a narrow clear zone. The interior is filled with a mass of chromatin in the form of granules or short rods. From many of these, filaments of varying thickness extend to nearby granules. The stouter filaments stain with iron-alum haematoxylin. Sometimes there is present a larger granule which has the appearance of a karyosome (pl. 22, fig. 90).

Extending from the blepharoplast to the nucleus there is a band-formed structure (pl. 22, figs. 86, 92). Besides this there appears to be a filament. The band is broadened close to the nucleus, is narrow in its middle portion, and on or close to the portion back of the blepharoplast is a subtriangular membrane (pl. 22, fig. 92) of which the apex often is curved backward in a hook form. This membrane, in about the same form and position, has been seen in many individuals. The posterior end of the band does not terminate at the anterior end of the nucleus, but extends beside it for at least a short distance; just how far could not be determined. The filament which lies close to this band is more slender.

The writer has been unable to determine the homologies of the structures described in the preceding paragraph. One is probably the rhizoplast, which in division (pl. 22, fig. 91) is doubled and connects the ends of the paradesmose to the blepharoplasts. It is not certain which of the two structures of the resting stage is the rhizoplast. The other structure is also present in the anaphase, located in the region between the two rhizoplasts. It may be the anterior part of the axostyle or parabasal body. The subtriangular membrane resembles the cresta of *Devescovina* in form, but not in position or staining reaction.

A well developed though slender axostyle is present (pl. 22, fig. 87). The filiform organelle shown by Grassi, extending from the region of the blepharoplast to and beyond the nucleus, probably is part of the axostyle, as Grassi suggested, though Grassi did not see the rest of the axostyle. The portion of the axostyle back of the nucleus is broadened and filled with small granules. Immediately behind the nucleus it is constricted; then, broadening again, it extends anteriorly on one side of the nucleus. Posteriorly the broadened portion tapers, then continues as a filament about 1μ thick to the end of the body, where it is pointed. There are no granules in the posterior portion of the axostyle.

Close to the nucleus and axostyle on the side toward the undulating membrane a long, slender rod-like structure can sometimes be seen, extending to about the middle of the body (pl. 22, fig. 87). This is not visible in Schaudinn-Delafield material, but it stains faintly reddish in material stained in iron-alum haematoxylin and counterstained in acid fuchsin. Probably it is the parabasal body.

In the cytoplasm anterior to the nucleus, and in the portion between the axostyle and the costa, there is a large number of small, often deeply staining granules, similar in size to those in the axostyle (pl. 22, figs. 86, 87). These granules are grouped in such a manner below the chromatic basal rod that irregular clear spaces are left, as described by Grassi.

A number of individuals show stages in outgrowth of the new costa (pl. 22, fig. 92). The structure shown by Grassi, located near the surface of the body and compared by him to a large flagellum, is probably the new costa.

As observed by Grassi, the surface of the body is traversed by numerous fine, longitudinal striations (pl. 22, fig. 88). In form and position, at least, these are suggestive of myonemes.

The paradesmose (pl. 22, fig. 91) is very broad, squared at the ends, and fibrillar like that of *Staurojoenina assimilis* (Kirby, 1926). It is more like the paradesmose of a hypermastigote than a trichomonad. From its ends somewhat band-like rhizoplasts extend to the blepharoplast. The chromosomes are long, rod-like bodies, apparently, in this one division figure seen, about nine in number.

The smaller trichomonads of 25–42 × 8–13μ are quite different in appearance from the large, typical forms. As transitional stages have not been found, it is possible that they represent a different species. As the material studied was limited, the status of this form—whether it is a distinct species or a stage in the development of *Pseudotrypanosoma giganteum*—must be left unsettled.

This form (pl. 22, fig. 93) has three anterior flagella, an undulating membrane like that of the large form, and a costa which is relatively more slender than that of the other. The nucleus, measuring 3 × 5 to 4 × 8μ, is relatively large and is broadly rounded at the ends. A moderate sized blepharoplast is present. The axostyle is stout, broadening gradually from behind the nucleus to the enlarged posterior part, then tapering and usually projecting from the body. It terminates in a short filament.

CLASSIFICATION AND DIAGNOSES

Family TRICHOMONADIDAE Wenyon, 1926

Several anterior flagella, one of which is often directed posteriorly and may or may not form an undulating membrane. Axostyle usually present as a rod-like structure, often projecting beyond the margin of the cytosome and pointed posteriorly. Parabasal body often, or usually, present as a straight, curved, serpentine or coiled, usually rod-formed structure attached to the anteriorly situated blepharoplast by a parabasal thread. Nucleus single, anterior.

Subfamily POLYMASTIGINAE subfam. nov.

Trichomonadidae with several anterior flagella, one of which is in most species directed posteriorly but is not adherent to the surface of the body. Short, straight, or curved parabasal body often present. Typical cresta of Devescovininae absent.

Polymastix has been selected as the basis for the name of this subfamily, because, although *Monocercomonas* has priority, the nomenclatural status of the genera named *Monocercomonas, Retortamonas,* and *Schedoacercomonas* by Grassi is much confused.

GENERA: *Polymastix, Monocercomonas,* (*Retortamonas*), (*Schedoacercomonas*), *Eutrichomastix, Tetramastix, Hexamastix, Tetratrichomastix, Janickiella* (?). The systematic position of *Janickiella* is uncertain; possibly it belongs in one of the other subfamilies.

Eutrichomastix Kofoid and Swezy, 1915

Three anterior flagella; one free trailing flagellum; axostyle not stainable with iron haematoxylin; short, rod-formed parabasal body; no costa or cresta.

Eutrichomastix axostylis sp. nov.

Elongate, ellipsoidal, or pyriform, axostyle projecting, length 8.1 (5–10.5) μ, width 2.7 (2–3.5) μ; three anterior flagella 7.5 (5–10) μ long, united proximally; trailing flagellum about thickness of an anterior flagellum, 13 (10–16) μ long; blepharoplast single, moderately large; rounded cytostome; axostyle very stout, enlarged and then tapering posteriorly, terminating in a short filament, no expanded capitulum anteriorly; one or two periaxostylar rings present; nucleus ellipsoidal, 1–1½ × 1½–2μ, large granular central chromatin mass surrounded by clear space beneath membrane.

Host.—*Nasutitermes* (*Subulitermes*) *kirbyi* Snyder. Barro Colorado Island, Canal Zone, Panama.

Subfamily DEVESCOVININAE subfam. nov.

Devescovinidae Poche, 1913

Usually (or always) three anterior flagella. One trailing flagellum which is stouter and usually longer than the others. Trailing flagellum may or may not be partially adherent to the body. Cresta near base of trailing flagellum, usually a small subtriangular, or sometimes rod-like, structure which becomes a large internal plate in some cases. Axostyle well developed. Parabasal body usually well developed, in most species coiled from one to many times around the axostyle back of the nucleus. Known only from termites.

GENERA: *Devescovina, Gigantomonas, Macrotrichomonas, Foaina, Parajoenia, Paradevescovina, Metadevescovina, Janickiella* (?).

These will be studied in a later paper of this series.

Subfamily TRICHOMONADINAE subfam. nov.

Several (two to five) free anterior flagella and one flagellum directed backward and adherent to surface of body, usually forming an undulating membrane. A costa is present beneath the undulating membrane. Axostyle usually well developed, sometimes enlarged into a capitulum anteriorly. Parabasal body usually, if not always, present; usually an elongated or rod-like structure varying from less than to several times the length of the nucleus. Nucleus single, containing chromatin granules which are concentrated into a central chromatin mass or more evenly distributed throughout the intranuclear region.

GENERA: *Trichomonas, Tritrichomonas, Pentatrichomonas, Ditrichomonas, Tricercomitus, Pseudotrypanosoma, Pentatrichomonoides.*

Trichomonas termopsidis Cleveland, 1925

Trichomitus (*Trichomitopsis*) *termitidis* Kofoid and Swezy, 1919.
Trichomonas termopsidis Cleveland, 1925, p. 312 (nom. nov.).
Trichomonas (*Trichomonopsis*) *termopsidis* Andrews, 1925.

Elongated when extended, rounded and flattened when contracted; length 43 (9–150 or 200)μ, breadth averaging about 14.5μ; cytostome large, elongated, crescentic; blepharoplast a single large granule; four anterior flagella about length of body; conical projection of cytosome at base of these flagella; undulating membrane large, free flagellum of moderate length; costa stout; axostyle with broad capitulum, stout trunk 1–2μ wide, posteriorly enlarged in a plumb bob form, or often not enlarged, terminating in a short filament up to 3μ long; endoaxostylar granules small, abundant in capitulum and trunk; parabasal body ½–1μ wide, up to 45μ or more in length, straight, curved, serpentine, or occasionally coiled loosely around axostyle; parabasal thread on surface, interior containing a row of granules; nucleus ellipsoidal or spherical; granular central chromatin mass surrounded by clear space; numerous granules, spherules, and particles of wood in cytoplasm; paradesmose not very stout; very numerous in hosts.

Hosts.—Termopsis nevadensis Hagen. California, Oregon. *Termopsis angusticollis* Hagen. California, Oregon. *Termopsis laticeps* Banks. Arizona.

Trichomonas barbouri sp. nov.

Broadly rounded anteriorly, rounded or tapering posteriorly; length 17.5 (11–24) μ, width 9 (6–14) μ; cytostome crescentic, moderate in size; blepharoplast moderately large, hemispherical or crescentic, apparently composed of several granules; near blepharoplast is a small parablepharoplast bar, from which a filament extends to the nuclear membrane; four anterior flagella 15–20 (25) μ long; conical projection of cytosome around base of these flagella; undulating membrane 1½–2μ high; costa moderately stout; axostyle expanded into a large capitulum anteriorly, tapering behind nucleus, lance head enlargement posteriorly, projecting from body only at extreme end; endoaxostylar granules few, arranged in a line or scattered; parabasal body large, rod-formed, bent at right angle beyond attachment to blepharoplast, one or two times nuclear diameter in length; nucleus broadly ellipsoidal or spheroidal, 4 (3–5½) × 5 (3½–6) μ; chromatin granules in large, dense central mass, usually surrounded by narrow clear space under membrane; cytoplasm contains particles of wood and various granules; paradesmose moderately stout, slightly enlarged at ends; fairly abundant in host, associated with various other polymastigote flagellates.

Host.—Kalotermes (*Glyptotermes*) *barbouri* Snyder. Barro Colorado Island, Canal Zone, Panama.

Trichomonas cartagoensis sp. nov.

Shape like that of *T. barbouri;* length 19.8 (14–29) μ, breadth 10.5 (6–14) μ; cytostome broad, crescentic; blepharoplast of moderate size; parablepharoplast bar present; four anterior flagella 15–20μ long; conical extension of cytosome at base of these flagella; undulating membrane fairly high, short free terminal flagellum; costa as in *T. barbouri;* axostyle with fairly large capitulum, trunk of uniform diameter, posterior enlargement and terminal filament; endoaxostylar granules few, scattered; axostyle projects at tip as in preceding species; parabasal body small, rod-formed, curved, hardly passing level of anterior end of nucleus; nucleus subspheroidal, 3 × 4–4 × 5μ, central mass with granules and masses of chromatin, usually surrounded by clear space; paradesmose moderately stout; chromosomes about six; particles of wood in cytoplasm; not abundant in host, associated with hypermastigote and other polymastigote flagellates.

Host.—Kalotermes (*K.*) *contracticornis* Snyder. Cartago, Costa Rica.

Trichomonas linearis sp. nov.

Body elongated, spindle shaped; length 15 (9–24) μ, breadth 4.25 (3–8) μ; cytostome small; blepharoplast large, at or near anterior tip, made up of several granules; four anterior flagella about 15μ long, emerge from body directly after leaving blepharoplast; undulating membrane rather low, posterior free flagellum 3–5μ long; costa very slender; axostyle rather stout, no enlarged capitulum, tapers to pointed posterior end without enlargement, terminates in short filament; endoaxostylar granules few, minute, indistinct; axostyle projects at posterior end for about 2.5μ; parabasal body not observed; nucleus very close to blepharoplast, elongate ellipsoidal 1.5 (1–3) × 4 (3–6) μ; interior of nucleus completely filled with small granules; granules in cytoplasm, but no wood observed; paradesmose slender; abundant in host, where it is the only protozoan present.

Host.—Orthognathotermes wheeleri Snyder. Barro Colorado Island, Canal Zone, Panama.

Trichomonas labelli sp. nov.

Moderately elongate, tapering posteriorly; length 8.4 (6–11) × 4.7 (3–7) μ; cytostome occasionally with posterior lip-projection; blepharoplast moderately large, at tip of body; four anterior flagella about 15μ long; undulating membrane fairly high, rather long free flagellum; costa slender; axostyle not stout, no distinct capitulum, tapers to pointed end without enlargement, terminates in short filament; axostyle projects for 2 to 7μ; parabasal body not observed; nucleus subspheroidal 1–½ (1–2) × 2 (1–3) μ; interior completely filled with rather large and few chromatin granules; in small numbers in host, where it is associated with large and small amoebae.

Host.—Mirotermes hispaniolae Banks. Barro Colorado Island, Canal Zone, Panama.

Tritrichomonas Kofoid, 1920

Characters like those of *Trichomonas,* but there are only three anterior flagella.

Tritrichomonas brevicollis sp. nov.

Rather broad, rounded posteriorly, undulating membrane curved around end; length 13.2 (10–17) μ, breadth 6.6 (4–8) μ; cytostome long, crescentic; blepharoplast a granule of fair size; three anterior flagella 20–25μ long; conical projection of cytoplasm about base of these flagella; undulating membrane well developed; costa rather slender; axostyle with moderately enlarged capitulum, stout trunk, slightly enlarged posterior end and terminal filament; axostyle projects at side of body for a short distance; parabasal body small, slender, not passing back to posterior end of nucleus; nucleus subspheroidal, about 3μ in diameter, contains a large, granular central mass and a clear space under membrane; cytoplasm contains granules, spherules, and wood; paradesmose fairly stout, enlarged at ends; in small numbers in host, associated with other polymastigote flagellates.

Host.—*Kalotermes* (*Calcaritermes*) *brevicollis* Banks. Barro Colorado Island, Canal Zone, Panama.

Tritrichomonas holmgreni sp. nov.

Form like that of *T. brevicollis;* length 16 (10–20) μ, breadth 5 (4–7) μ; cytostome and blepharoplast like those of preceding species; three anterior flagella about 20μ long; conical projection of cytosome about base of these flagella; undulating membrane of moderate height, no free flagellum; costa rather slender; trunk of axostyle fairly stout, tapers without marked enlargement, terminates in a short filament; parabasal body slender, short, not passing back of posterior end of nucleus; nucleus subspheroidal, about 2 × 3μ, space within membrane entirely filled with numerous chromatin granules (thus differing from *T. brevicollis*); cytoplasm contains granules and fragments of wood; fairly numerous in its host, associated with other polymastigote flagellates.

Host.—*Kalotermes* (*Neotermes*) *holmgreni* Banks. Panama.

Pentatrichomonoides gen. nov.

Five anterior flagella and undulating membrane present; axostyle very slightly developed; parabasal body somewhat fusiform or sac-formed; nucleus at some distance from the anterior blepharoplast; innominate filament present.

Genotype.—*P. scroa* sp. nov.

Pentatrichomonoides scroa sp. nov.

Body stout, squarely or obliquely truncate posteriorly, undulating membrane spiralled and passing around posterior end, body often grooved beside membrane; length 29.4 (18–45) μ, breadth 4.3 (6–15) μ; cytostome not observed; blepharoplast elongated, crescentic, placed transversely or obliquely; innominate filament from blepharoplast passing to short distance behind nucleus; five anterior flagella 25 (18–30) μ long; usually adherent to one another for most of length; small conical projection of cytosome at base of this whip; undulating membrane rather low, no free posterior flagellum; costa rather slender; axostyle very slender, usually invisible; parabasal body short, fusiform, pyriform, or rounded, attached to blepharoplast by peduncle, often flattened on one side, large, deeply staining granules sometimes visible in stoutest part; nucleus at about a quarter of the body length from the anterior end, close to surface of body; subspheroidal, about 3 (2–5) μ in diameter, one or two chromatic masses near center and numerous chromatin granules under membrane; cytoplasm contains various granules, rods, and spherules, but wood not observed; paradesmose band-formed, unusually broad for a trichomonad; abundant in its hosts, associated with various other polymastigote flagellates.

Type host.—*Kalotermes* (*Cryptotermes*) *dudleyi* Banks. Canal Zone, Panama.

Additional host.—*Kalotermes* (*Lobitermes*) *longicollis* Banks. Taboga Island, Panama.

Pseudotrypanosoma Grassi, 1917 emended

Large, elongated; three anterior flagella; undulating membrane; axostyle slender; band-like structure between blepharoplast and nucleus; nucleus at some distance from the blepharoplast; striations (myonemes?) near surface of body; parabasal body long, narrow.

Pseudotrypanosoma giganteum Grassi, 1917 emended

Large, elongated forms 55–111μ long (Grassi's figures) or 145–205 x 20–40μ (present measurements); cytostome not observed; blepharoplast large, flattened, or concave on one side; convex, plate-like structure in cytoplasm anterior to blepharoplast, connected to blepharoplast; three anterior flagella about 30μ long; undulating membrane relatively low; costa very stout; axostyle broad back of nucleus, tapering to a slender trunk, not projecting from body; endoaxostylar granules abundant in portion just back of nucleus; parabasal body slender, very long (this structure somewhat doubtful); nucleus at some distance (20–25μ) from blepharoplast, elongated, 13–20 x 3–6μ; interior occupied by a mass of granules; band-like structure between blepharoplast and nucleus; subtriangular, apically hooked membrane on or close to this band; cytoplasm containing a large number of small granules; longitudinal or oblique striations (myonemes?) on or near surface; paradesmose very broad, band-formed, squared at ends, fibrillar; abundant in hosts, associated with a number of hypermastigote flagellates.

Smaller, more typical trichomonads also found in *Porotermes grandis;* 25–42 x 8–13μ; three anterior flagella; nucleus 3 x 5 to 4 x 8μ, close to blepharoplast; axostyle stout, projecting, terminating in a short filament. Structure that of a typical *Tritrichomonas,* doubtfully referred to this species at present.

Hosts.—*Porotermes adamsoni* (Froggatt). Australia. *Porotermes grandis* Holmgren. Victoria, Australia.

DISCUSSION

SIZE

The described flagellates of the subfamily Trichomonadinae, exclusive of those in termites, range in length from 3 to 30μ, and in one case to 35μ. One of the smallest species is *Trichomonas ortyxis,* from the valley quail, with a mean length of about 5.5 and a range from 3.5 to 7.5μ (Hegner, 1929). One of the largest is *Trichomonas alexeieffi,* which ranges in length from 15 to 30μ and in width from 12 to 18μ (Grassé, 1926). Parisi (1910) records 35μ as the maximum length of a *Trichomonas* from the axolotl. The range in length of the Trichomonadinae from termites, not including *Tricercomitus,* is from 6 to 205μ. Some of the Trichomonadinae from termites are small. Of the fifteen described species, eight have a maximum length of 20μ or less. The smallest is *Trichomonas labelli,* ranging from 6 to 11μ long. Three others have a maximum length of less than 30μ. Giantism is exhibited only in four species, *Pentatrichomonoides scroa,* 18 to 45μ; *Ditrichomonas termitis,* 30 to 88μ; *Trichomonas termopsidis,* 9 to 150 or 200μ, and *Pseudotrypanosoma giganteum,* up to 205μ long. Giantism is not a general phenomenon in this subfamily in termites, but it is known to occur only in these insects.

FORM AND MOVEMENTS OF BODY AND AXOSTYLE

Two types of form changes have been described in trichomonad flagellates. As the body is not enclosed by a thick pellicle, as it is in *Devescovina,* changes of form are easily accomplished. In animals under abnormal conditions movements described as amoeboid have been observed by Dobell (1907) in *Eutrichomastix serpentis* and (1909) in *E. batrachorum,* by Wenyon (1907) in *Tritrichomonas muris,* by Kuczynski (1914) in old preparations of *T. caviae,* by Reuling (1921) in *Trichomonas vaginalis,* by Cutler (1919) in *Ditrichomonas termitis,* and by others. During this moribund activity protuberances suggesting pseudopodia appear from various parts of the surface. It is purely a degeneration phenomenon. The writer has observed it in moribund *Trichomonas termopsidis* and has described (1926) similar degenerative processes in *Staurojoenina assimilis.*

The other type of form change is normal, and appears to occur in a number of Trichomonadinae. In this paper, it has been described in *Trichomonas termopsidis* (p. 183). The body contracts vigorously, bending ventrally, and then straightens out again. In the process costa and axostyle are bent, the former taking almost the form of a circle in some cases. Such movements have been observed in *Trichomonas caviae* by Kuczynski (1918). The body bends ventrally, the bent forms being flattened, all locomotor organs continue active, and the animal quickly passes from this form into the swimming form ("Schwimmform"). Kofoid and Swezy (1923) have described such movements in *Pentatrichomonas ardindelteili,* in which progression is interrupted by brief intervals of circling movements in short arcs, a quick, spasmodic contraction preceding the interruptions in direction. Reuling (1921) mentions similar form changes in *T. vaginalis.*

Among the species described in this paper, this change of form has been observed only in *Trichomonas termopsidis* in living material, but the shapes of the fixed individuals of all species except *Trichomonas linearis* and *Pentatrichomonoides scroa* indicate that this contraction occurs. But *T. linearis* and *P. scroa* have always been found in a linear form, so that in them it is probable that the bending does not occur. Apparently it does not take place in *Eutrichomastix axostylis.*

What causes this bending of the body? Is it the active bending of the axostyle, the active contraction of the costa, the contraction of the cytoplasm of the body, or the synchronous action of two or all of these? The question cannot be answered from the evidence now at hand, but it may be worth while to discuss certain facts bearing upon the problem.

When the costa is very stout, as in *T. termopsidis* and *Pseudotrypanosoma giganteum,* it is conceivable that its contraction may cause the bending of the body. But in most trichomonads it is slender. It is highly improbable that so slender a costa as that in *P. ardindelteili* and *T. vaginalis* could be the sole cause of the bending.

No myonemes have been described in *Trichomonas.* In *Pseudotrypanosoma giganteum* there are longitudinal, myoneme-like striations, which possibly are contractile. It may be that in other trichomonads there are similar but unrecognized contractile elements in the cytoplasm. Without these, however, the contractile property of the cytoplasm itself could account for the bending, at least in most cases.

It is noteworthy that the degree of development of the axostyle is without relation to the habit of bending the body. In the two species described above which appear not to have this activity, *T. linearis* has a very well developed axostyle while *P. scroa* has a mere vestige of this structure. Of those which do contract in this way, *T. termopsidis* has a large axostyle, *P. ardindelteili* and *T. vaginalis* have slender axostyles.

It is evident that the flexible axostyle must bend with the body. It has been shown that the bending of the axostyle takes place chiefly in a portion back of the capitulum, and here the structure is often flattened (*T. termopsidis, Hexamastix laticeps*). That it is actively contractile and alone causes bending of the body is very unlikely; there is no structural basis in the axostyle for such power of vigorous contraction, and certainly none where it is very slender as in *P. ardindelteili.*

In certain large individuals of *Trichomonas termopsidis,* most of which are in a prophase of division and have two costas, the posterior portion of the body becomes tail-like, after having lost some cytoplasm, with a very small amount of cytoplasm covering the posterior portions of the costas and axostyle. This tail-like portion may perform vigorous bending movements. There is so little cytoplasm present that it seems likely that the movements are due to the axostyle or the costas. When in normal condition these large individuals are of even diameter along the longitudinal axis, and they perform similar bending movements.

Much discussion has been devoted by various authors to the question of the activity of the axostyle. Grassé (1926) agrees with most investigators in his doubt that the axostyle is motile of its own accord, and his belief that, while it is sometimes displaced, even violently, in the plasma, it is a passive organ. Kofoid and Swezy (1915) claim active motility for the axostyles of *Tritrichomonas augusta* and *Eutrichomastix serpentis.* In his criticism of their paper, Kuczynski (1918) states that they probably observed the bending which he described, and misinterpreted this as axostylar lashings. Alexeieff (1924) agrees with Kofoid and Swezy and chooses to regard the axostyle as an internal flagellum. He observed bending of the "axostyles" in *Octomitus intestinalis,* though he admits the possibility that the apparently active bending is actually preceded by contraction of the surrounding cytoplasm, and is passive. He believes that the axostyle serves as a rudder, and when there are posterior flagella these aid in progression in the manner of a stern oar of a boat. One is not justified in applying to the Trichomonadinae the results of observations on the so-called axostyles of *Octomitus,* for in that flagellate the "axostyles" are the intracytoplasmic parts of the posterior flagella. Andrews (1925) states that the axostyle of *Trichomonas termopsidis* "appears to function as an internal paddle, continually churning up the contents of the body." The writer has observed no such movements of the axostyle of *Trichomonas termopsidis.* In *Pyrsonympha* the axostyle does perform undulating movements within the cytoplasm, but it is not exactly homologous with the axostyle of *Trichomonas.*

CYTOSTOME

The cytostome has been found in many species of Trichomonadinae, as well as in *Eutrichomastix.* In some it has not been reported and may be absent, although it is possible to examine much material of some species without recognizing the structure. Dogiel states that it is absent in *Trichomonas termitidis* and Swezy (1915) makes the same statement in regard to *Trichomitus parvus.* Bernstein does not report it in *Trichomonas vermiformis.* It seems to be absent in *Pentatrichomonoides scroa* and *Pseudotrypanosoma giganteum.* It is, however, a very characteristic feature of most of the Trichomonadinae. A lip-like prominence at the posterior border of the cytostome has been observed in *Trichomonas macrostoma* (Dogiel, 1916), *Trichomonas sanguisugae* (Alexeieff, 1914), as well as in *Trichomonas labelli.* It is not a persistent feature and is not present in all individuals of the species, but its formation is probably more typical of some species than of others. Grassé (1926) believes that the cytostome is not functional, certainly not in saprozoic species like *T. augusta* and *T. lacertae,* in which it is well developed. The writer has no evidence from observation that the cytostome actually functions as the region for ingestion of solid food, though the presumption has been that it does. On the one hand, *P. scroa* and *Pseudotrypanosoma giganteum,* which ingest solid food, seem to lack cytostomes, and on the other hand, cytostomes are present in some species (*T. augusta, T. linearis*) which do not ingest solid food.

BLEPHAROPLAST

The blepharoplast is often shown as a single, spherical granule, and no doubt it is a single granule in many species. In some species, however, it has been shown that the blepharoplast is made up of several granules closely aggregated in a common matrix, so that when heavily stained it appears as a single mass. This is true of *Trichomonas vaginalis*, *T. floridanae*, *T. gallinarum*, *T. eberthi*, *T. linearis*, *T. barbouri*, and others. Kuczynski (1918) notes that the size of the blepharoplast varies greatly; that in some cases a central stainable mass is surrounded by a lighter mantle, and in others distinct granules are visible. He is referring chiefly to *T. muris*, *T. augusta*, and *T. caviae*. No doubt more critical destaining would show that the apparently single blepharoplast in some cases is composed of several basal granules.

In some species several granules not aggregated into a single blepharoplast are reported. Two blepharoplasts are reported in *Trichomonas anseri* (Hegner, 1929), *Pentatrichomonas ardindelteili* (Kofoid and Swezy, 1923), and in other species. Hinshaw (1926) finds three blepharoplast granules arranged in a row in *Trichomonas buccalis*. In certain species the costa and marginal flagellum end in a separate granule from that to which the other structures connect. This arrange- is shown by Cutler (1919) in *Ditrichomonas termitis*, in which both granules are unusually large, and by Duboscq and Grassé (1924) in figures of *Trichomonas trypanoides*, in which one granule is smaller than the other. The most unusual arrangement is shown by De Mello (1923) in a species of *Tritrichomonas* from termites. In this there are two separate blepharoplasts, one single for the costa, marginal flagellum, and the nuclear rhizoplast; the other composed of three separate granules, one for each anterior flagellum. Some of his figures show these granules quite separate (fig. A, 1); others show only one large granule.

In each of the trichomonads described in this paper the blepharoplast is a single mass, which in *T. barbouri* and *T. linearis*, at least, is composed of several basal granules imbedded in a common matrix. In *T. termopsidis* and several other species the blepharoplast is spherical, as is the case in practically all species reported in the literature. But in *T. barbouri* the structure is hemispherical or crescentic, with the organelles and flagella joined to various definite portions. In *P. scroa* it is elongate crescentic, and in *Pseudotrypanosoma* *giganteum* the large granule is flattened or concave on one side. Gineste (1913) shows a large, crescentic blepharoplast in *T. caviae,* but later authors show a rather small, round granule or pair of granules in that flagellate. In it there is often a row or aggregation of granules, sometimes forming a rather dense body, in the position in which Gineste's blepharoplast is located. This is not constant, and it appears to be part of a group of granules which extends beyond the nucleus. Possibly this was mistaken by him for the blepharoplast, as it sometimes has the form he shows.

RHIZOPLAST

Most authors do not show, and some state that they did not find, a rhizoplast from the blepharoplast to the nuclear membrane in species of Trichomonadinae, but there is no doubt that it is present, at least in some species. Grassé (1926) found rhizoplasts in several species. In *Trichomonas termopsidis* the rhizoplast has been seen by Kofoid and Swezy, Andrews, and the writer, and it is a characteristic structure. Some writers (Fonseca, 1915; De Mello, 1923) have shown the rhizoplast extending to the karyosome of the nucleus, but this is probably incorrect. A centrosome on the nuclear membrane has been shown by Kofoid and Swezy in *Pentatrichomonas ardindelteili,* but not by the same authors in *T. augusta* and *T. termopsidis.* Hinshaw found this in *T. buccalis.* It has not been found by other investigators in various trichomonads.

Kuczynski (1914) believes that what has been called the rhizoplast is a residuum of the spindle. Grassé (1926) suggests that perhaps the rhizoplast of the adult *Trichomonas* represents the remains of the paradesmose, a portion of which, becoming applied to the nucleus, puts it in relation to the blepharoplast. The writer cannot agree with this idea as far as *Trichomonas termopsidis* and a number of other species studied in this paper are concerned. In these the rhizoplast may be the strand which connected the centrosome (or the end of the paradesmose) and the blepharoplast during division. The paradesmose, and with it the supposed centrosome, disappear.

Grassé (1926) notes that after osmic fixation the rhizoplast of *Proteromonas* does not stain, but appears as a clear band. In *T. termopsidis* the writer has observed that the rhizoplast does not stain with iron haematoxylin after osmic vapor, but does after Schaudinn's fluid.

PARABLEPHAROPLAST BAR

The parablepharoplast bar has been seen in *Trichomonas barbouri* and *T. cartagoensis* of the species described in this paper, but has not been recognized in the others. In *T. termopsidis* and *P. scroa,* at least, the structure is absent. In *T. barbouri* the filament extending to and along the nuclear membrane has been very clearly seen.

Grassé (1926) has shown a structure which appears to be similar to this in *Trichomonas batrachorum* (his pl. 16, fig. 229) and in *T. augusta* (his pl. 17, fig. 274), but he has not commented upon it. In the latter species, on a slide prepared from *Ambystoma* by Mr. A. E. Noble, the writer has seen a parablepharoplast bar of the same relative size and in the same position as that of *T. barbouri.* This was distinct, deeply stained, and easily recognizable in all specimens. It has been searched for in *T. muris* and *T. caviae* without success.

It may be that this structure is part of the cytoendoskeleton of the body; perhaps it is related to the axostyle. The small bar is located on the border of the cytostomal portion of the body, where it appears to be firmly fixed, and the filament which extends from it to the nucleus, in *T. barbouri* at least, might be suspensory.

FLAGELLA AND UNDULATING MEMBRANE

The anterior flagella arise from the blepharoplast, may or may not pass through an appreciable zone of cytoplasm before becoming free, and often adhere together in the proximal portion. They adhere for a good part of their length in most of the species here described, and this adhesion has been reported in *T. termitidis, T. vermiformis,* and *T. trypanoides.* In *P. scroa* there is normally a whip of five flagella, which are separate only at the distal end. The extreme case is reached in *Hexamastix claviger* and related species, and in the elongated forms of *Tricercomitus termopsidis,* in which the flagella are completely united into a stout whip (Kirby, 1930). After fixation the flagella are often more or less completely separated.

In most of the species described above the cytoplasm extends around the base of the group of anterior flagella in a conical prominence. Usually in trichomonads the anterior end is shown rounded off and the flagella emerge abruptly. This is no doubt true in many cases, but since so many of the forms in termites have the conical extension, it seems likely that this exists more commonly in other forms than the records lead one to expect. Hinshaw (1926) states that in *T. buccalis* "the anterior tip of the body forms a cone-shaped projection of the protoplasm" at the base of the anterior flagella. Grassé (1926) shows this in *T. alexeieffi* and *T. lacertae,* but not in other species. Andrews (1925) states that "the cytoplasm is usually raised into a small hillock" at the point of emergence of the anterior flagella in *T. termopsidis,* though only one of his figures shows any indication of this. When such a prominence is present, it moves with the lashing of the whip of flagella.

A free portion of the marginal flagellum is quite characteristic of trichomonads. Sometimes this is of considerable length. It is not present in all species, however. In most of the species described above it appears to be shorter than in many trichomonads, and it is absent in *P. scroa* and *Tritrichomonas holmgreni.*

COSTA

In larger species of Trichomonadinae the costa is usually relatively, as well as actually, stouter than in smaller species, but in species of similar size its degree of development varies. Greatly enlarged costas occur in the giant *Pseudotrypanosoma,* in *Ditrichomonas termitis,* and in *Trichomonas termopsidis.* In *P. giganteum* the costal development is independent of the development of the undulating membrane, for in that flagellate the undulating membrane is low. *P. scroa* is an exception to the apparent relation of the costal development to the size of the body, for this species is rather large but has a slender costa.

It has been observed that occasionally the costa breaks up into a series of granules. This has been reported by Alexeieff (1914) in *T. sanguisugae* and *T. augusta,* by Faust (1921*a*) in *T. hominis* and (1921*b*) *T. flagelliphora,* and by De Mello (1923) in a species of *Tritrichomonas* from termites. An instance of this was noticed in *Trichomonas barbouri,* but it is unusual. Probably it is a degeneration phenomenon.

The costa usually stains deeply with iron-alum haematoxylin. It has been observed that in certain material of *T. termopsidis* fixed in Schaudinn's fluid, the costa failed to stain with this dye, though the nucleus was well stained. The costa stains not at all, or feebly, in Delafield's haematoxylin. In Mallory's triple stain the costa stains red, unlike the other permanent structures of the body. Evidently the chemical nature of the costa is different from that of the flagella, blepharoplast, parabasal body, or chromatin. In its reactions to Heidenhain's and Delafield's haematoxylin it resembles the paradesmose, and Alexeieff (1928) shows it stained with Giemsa like the paradesmose in *T. augusta.*

Is there a structure which can be homologized with the costa in *Eutrichomastix?* Most authors find no such structure. But in *Eutrichomastix gallinarum* Martin and Robertson (1911) find a slender chromatic line in the position of a costa and state that a line grows out anew in one of the daughter individuals. Perhaps in their descriptions of this species and this structure they were dealing with *Tritrichomonas eberthi* in which the membrane was detached or obscure, as Grassé (1926) has pointed out. Except for this, one can distinguish between a *Eutrichomastix* and a *Tritrichomonas* with detached membrane by the absence or presence of a costa. The writer can see no reason for the contention of Grassé that *Eutrichomastix* is a stage in the development of *Trichomonas* (or *Tritrichomonas*), that in some hosts it does not develop into the "adult form," and that it should be reduced to a synonym of the latter genus.

AXOSTYLE

Grassé (1926) calls attention to the existence of two forms of axostyle in *Trichomonas.* In one, the caliber is nearly uniform, without expansion in the region of the nucleus; in the other, there is a flattened anterior dilation or capitulum. In most described Trichomonadinae, the axostyle has no capitulum, but it is well developed in *Tritrichomonas augusta* from amphibia, and in *Trichomonas termopsidis, T. barbouri,* and *T. cartagoensis* from termites. In *T. augusta* the capitulum is a flat, spatulate, gradually broadened head of the axostyle, and the trunk extends directly from its posterior portion. In the other three species the capitula are broader, there is more or less of a bend where the trunk emerges, and there is a more distinct demarcation between the trunk and head. Bernstein (1928) shows an unusual axostyle in *Trichomonas vermiformis.* It is said to begin behind the nucleus, where it is very much broadened. Probably this is the posterior part of a capitulum, and actually it extends anteriorly to the blepharoplast.

Besides these two general groups of axostyles as to form, there are two kinds of axostyles as to stainability. In most species, the axostyle remains unstained, except for the sheath and often the inner granules. But some slender axostyles, as those of *Trichomonas buccalis* (Hinshaw, 1926), *T. didelphidis, T. cynomysi, T. felistomae,* and *T. canistomae* (Hegner and Ratcliffe, 1927*a, b*) are shown as deeply stained with iron haematoxylin. In no case are tubular axostyles, like those of Trichomonadinae reported from termites, stainable throughout. Apparently when the substance is concentrated into a very small caliber, the whole may stain as does the axostylar sheath.

The tubular axostyle tapers, often rather abruptly, at the posterior end, and in most species is shown as ending in a point. The posterior portion of the trunk usually projects from the cytoplasm. In trichomonads not in termites, the end of the axostyle seldom is described as enlarged. Hegner (1929) shows such an enlargement in *T. floridanae*, Cunha and Muniz (1927) show it in *Eutrichomastix aguti,* and often there is some enlargement in *T. muris.* In several of the Trichomonadinae of termites, however, the axostyles have ends conspicuously like a lance head or plumb bob. This is the case in *Trichomonas barbouri, T. cartagoensis,* and *T. vermiformis,* and is frequently true of *T. termopsidis.* A similar enlargement occurs in certain species of *Spirotrichonympha*, *Hexamastix*, in some of the Oxymonadidae, and in *Devescovina polyspira Lewis* (MSS).

In all the species described in this paper which possess well developed axostyles, these end in terminal filaments of moderate length. The terminal filament was observed in several species of *Hexamastix* from termites (Kirby, 1930). It seems, from the writer's observations, to be quite general in the Trichomonadidae. But the terminal filament is seldom shown in figures of *Trichomonas.* Kuczynski (1914) states that when the axostyle comes loose from adherence to the substratum, one often gets the impression that a short thread, to which bacteria adhere, is drawn out from the end. He shows the filament in some of his figures of *T. augusta* and in one of *T. caviae.* According to the writer's observations, the filament is not "drawn out" in this way, but is a persistent structure. It is present on axostyles enclosed completely in the cytoplasm (pl. 14, fig. 11). Grassé (1926) shows the filament in a number of species of *Trichomonas, Eutrichomastix,* and *Hexamastix.* Knowles and Gupta (1929) show a rather long filament at the end of the axostyle of a *Trichomonas* from the porcupine. Otherwise, the writer has not seen it described or figured. Often it is not shown in figures of *T. augusta,* which has a long terminal filament. It is probable that this structure is of general occurrence and has been overlooked in many cases. If the axostyle is used as an organ of fixation, the terminal filament would be of great assistance in this.

Trichomastix salina, described from continental saline water of high concentration by Entz in 1904, has been mentioned by Grassé (1926) as a free living flagellate which has an axostyle. Through the kindness of Dr. L. B. Becking of the Hopkins Marine Station the writer has been able to examine this flagellate, and finds that the structure which is supposed to be an axostyle is a ventral groove which Entz himself did not call an axostyle. The flagellate does not belong in either the genus *Trichomastix* or *Eutrichomastix.* It has four flagella, two directed posteriorly and two anteriorly.

Periaxostylar rings appear to be absent from all of the trichomonads described in this paper except *Eutrichomastix axostylis.* They have been described in a number of species, however, including *Tritrichomonas augusta, T. batrachorum,* and *T. muris.* Some authors have observed the optical sections of the rings as granules beside the axostyle. The rings are very conspicuous in *T. muris,* located at the beginning of the posterior enlargement, and they are present in the same position when this part of the axostyle projects from the body, as in *E. axostylis.* The rings, being adherent to the axostyle and not attached in the cytoplasm, could not serve as an anchor, as Reuling (1921) suggests they may.

The endoaxostylar granules are characteristic structures in the larger, tubular axostyles, but they are not always present. They are most conspicuous in *Tritrichomonas augusta.* In certain species of *Hexamastix, Eutrichomastix,* and Trichomonadinae from termites they are well developed. In the staining reactions which have been tested (p. 186) they behave like the costa, except in staining with Lugol's solution. They vary some in number in different individuals of species which possess them, but on the whole they are constant. Alexeieff (1917*c*, 1924, 1928) regards these granules as mitochondria, but their staining reactions are quite unlike those usually supposed to be characteristic of mitochondria and it seems to the writer that there is little evidence for putting them in that category.

Alexeieff (1917*b*, 1928) finds a spiral arrangement of the endoaxostylar granules in *T. augusta.* He relates these to the parasome (parabasal body), comparing their spiral to that of the parasome of *Devescovina,* and appears (1917*b*) to believe that some of them, by fusing, may form the parasome. With this conclusion the writer cannot agree. They are entirely distinct from the parabasal body in every respect.

The axostyle and the endoaxostylar granules apparently contain glycogen, as indicated by the fact that these granules and the entire axostyle stain brown in Lugol's solution. This has been observed in *Tritrichomonas augusta* (Alexeieff, 1929) and in *Trichomonas termopsidis* (p. 187).

PARABASAL BODY

The parabasal apparatus of the Trichomonadidae consists of a single parabasal body attached to the blepharoplast. In position the parabasal body is usually dorsal, and in all cases which the writer has observed it lies, at least in its proximal portion, between the costa and the nucleus. Grassé (1926) says that it is ventral in *Tritrichomonas batrachorum.* In form the body is spherical, drop-shaped, or bacilliform in *Tricercomitus;* sac-formed or club-shaped in *Hexamastix;* rod-shaped or club-shaped in most of the Trichomonadinae. In most cases the body is rather short, but it is long in some of the Trichomonadinae and Devescovininae. Among the trichomonad flagellates it reaches its greatest development in certain of the flagellates in termites—*Trichomonas termopsidis, Ditrichomonas termitis,* and the Devescovininae. The longer parabasals show a tendency to coil about the axostyle. This is indicated in *Trichomonas termopsidis,* and is a characteristic feature of the Devescovininae.

The parabasal body is not of simple, homogeneous structure. This has been shown by Alexeieff, Duboscq and Grassé, Grassé, and in the descriptions in this paper. Janicki (1915) distinguished the parabasal thread as a more deeply staining filament situated at the periphery of the parabasal body, or bearing the numerous rod-formed or leaf-formed "parabasalia" of *Joenia.* Cutler (1919) described the thread in the parabasal body of *Ditrichomonas termitis* as running down the middle, but it is probably not actually in the center of that body. The present author (Kirby, 1926*b*) described the thread as running through the parabasal body of *Devescovina lemniscata,* but it is on the periphery. This thread is on the edge of the spherical parabasal of *Stephanonympha nelumbium* (Kirby, 1926*b*), of the cord-like parabasals of *Trichonympha chattoni* (Duboscq and Grassé, 1927), of this body in *Devescovina hilli* (Duboscq and Grassé, 1927), and *D. polyspira* (Lewis, MSS.). This is its situation in *Trichomonas termopsidis.* It seems, then, that the peripheral situation of the parabasal thread is general.

Duboscq and Grassé (1927) distinguish the so-called thread as a chromophile border of the parabasal body, which serves as a skeletal support for the more labile, chromophobe substance. Except for this border, the outlines of the parabasals of *Devescovina hilli* and *Trichomonas chattoni* appear to be rather uneven. In *Trichomonas termopsidis,* however, there is a very definite outline, the parabasal appearing to be enclosed in a sheath. This is also the case, according to the writer's observations, in *Devescovina.*

According to Duboscq and Grassé the chromophobe substance consists of a series of achromatic spherules in a matrix which stains more or less with iron haematoxylin. Grassé (1926) finds a similar structure in *Tritrichomonas batrachorum,* and both these authors believe that the vesicles may be extruded into the cytoplasm. Appearances suggesting this structure have been seen in the parabasals of various trichomonads and Devescovininae, but the writer has no evidence that vesicles are released into the cytoplasm. In *Trichomonas termopsidis* stained with Delafield's haematoxylin after different fixatives, including Schaudinn's, mercuric chloride, formalin vapor, and Champy's fluid, a different and very well defined structure has been seen. This is a row of more deeply staining granules in a more lightly staining, or unstained, matrix, the whole enclosed by a sheath. In *Metadevescovina debilis* sometimes the structure is similar, except that the granules are larger blocks, sometimes the more deeply staining substance is continuous at the periphery so that the more lightly stained substance is enclosed as a series of vacuoles. The thread or chromophile filament is not differentiated in Delafield stained parabasals.

A granular structure in the parabasal body has been shown by other writers. Alexeieff has noted this in several species and he believes that the body is formed by the fusion of a series of cytoplasmic granules, which, according to him, are mitochondria, or even by fusion of some of the endoaxostylar granules. Grassé (1926) shows parabasal bodies, containing granules arranged much like those in *T. termopsidis,* in *T. batrachorum* (his pl. 16, fig. 248), and in *Monocercomonas melolonthae* (his text fig. 34).

The structure of the parabasal of *Pentatrichomonoides scroa* is interesting in this connection. In this body, which is only slightly elongated, the substance which stains more readily with Delafield's haematoxylin is concentrated in a single, rather large granule. Alexeieff (1924) mentions the occurrence in certain cases of a karyosome-like structure in the parabasal body.

In its chemical nature the parabasal body is unique and unlike other structures of the body. The reactions of the parabasal of *Trichomonas termopsidis* to certain fixatives and stains have been reported on page 189. In many trichomonad flagellates Delafield's haematoxylin stains the body after all fixatives the writer has used, without relation to the amount of acetic acid, but as a rule, only part of the substance is stained, as the granules of *T. termopsidis* and the blocks of *Devescovina*. In *Tricercomitus* the body stains uniformly with this dye; that is, the Delafield-staining substance composes the entire parabasal. When Delafield's does not stain the structure, as in certain species of *Hexamastix* and *Trichomonadinae,* it is probably because this substance is smaller in amount or absent. Perhaps, as suggested by Alexeieff (1928), richness in lipoids determines the staining properties. He observes that structures supposedly rich in lipoids are strongly siderophile. In that case the Delafield-staining substance of the parabasals of trichomonads is rich in lipoids. He remarks that substances poor in lipoids are feebly siderophile and do not stain in Delafield's.

Obviously it is incorrect to say that Schaudinn's fluid or acetic acid dissolves the parabasal body. It is true only that after Schaudinn's fluid or the vapor of acetic acid, as after concentrated $HgCl_2$ and formalin vapor, the parabasal does not stain with iron-alum haematoxylin. After osmic acid and chromic acid the body does stain with iron haematoxylin but its true structure is poorly shown.

The parabasal body differs from the costa in its reaction to Mallory's stain, the Champy-Kull method, and Delafield's haematoxylin. Toward the first two of these it behaves like the blepharoplast. The blepharoplast usually, but not always, stains readily with iron-alum haematoxylin after Schaudinn's fluid, while the parabasal does not. Cunha and Muniz (1928) report that the parabasals of *Tritrichomonas batrachorum* and *Tritrichomonas muris* do not stain with Feulgen's stain, while that of *Herpetomonas* does. The paradesmose does not stain with Delafield's.

NUCLEUS

The nucleus in trichomonad flagellates is located usually near the anterior end, not far from, if not actually against, the blepharoplast. There are exceptions to this in *Pentatrichomonoides scroa* and *Pseudotrypanosoma giganteum*. The spheroidal or broadly ellipsoidal shape is the rule, but in *Pseudotrypanosoma* the nucleus is much elongated. A delicate but well defined nuclear membrane is present.

As regards general structure, two types of nuclei have been reported in trichomonad flagellates. In the first, the more common type, the interior is completely filled with small chromatin granules and there is often a single, larger, peripherally located karyosome. There is a modification of this type in some species, as *T. termopsidis, T. barbouri, T. cartagoensis, Tritrichomonas brevicollis,* and *Pseudotrypanosoma giganteum,* in which there is a narrow, clear space between the membrane and the large, central chromatin mass. In the second type of nucleus there is a relatively small central chromatin mass, sometimes called a karyosome, surrounded by a broad, clear area; and there is a shell or layer of granules encrusting the membrane. This type of nucleus is suggestive of that in certain amoebae. The writer has not observed it in any Trichomonadinae of termites, but it has been described in *Trichomitus parvus* (Swezy, 1915), *Pentatrichomonas ardindelteili* (Kofoid and Swezy, 1923), *Trichomonas aragaoi* (Cunha and Muniz, 1927), *Trichomonas cynomysi* (Hegner and Ratcliffe, 1927), and others.

These two types of nuclei have sometimes been found in the same species. Hinshaw (1926) states that he has found rarely nuclei in *Trichomonas buccalis* with a central karyosome, peripheral ring, and spoke radii. The nuclei of *Eutrichomastix batrachorum* and *Tritrichomonas batrachorum* typically are filled with chromatin granules, but Dobell (1909) states that in the cysts of both of these, and also in a *Tritrichomonas* said to be about to encyst, the nuclear chromatin concentrates in the center as a large karyosome and also collects just under the membrane. In *Trichomonas gallinarum,* in which the nucleus is ordinarily filled with granules, Martin and Robertson (1911) report some in which the chromatin is almost all condensed into a large, central karyosome. Two types of nuclei like these have been observed in *Hexamastix termopsidis* and *H. laticeps* (Kirby, 1930).

CYTOPLASMIC GRANULES AND MITOCHONDRIA

Grassé's classification of the cytoplasmic inclusions of trichomonads is a valuable one. He recognizes five categories of these: paracostal granules; frontal granules; chromatic granules in the cytoplasm; mitochondria, stainable with Janus green; and vacuoles, stainable with neutral red, cresyl blue, and methylene blue. Of the vacuoles, some are alimentary, containing food in the process of digestion, and some are metaplasmic, formed by the activity of protoplasm. Faust

(1921*a*, *b*) reported pulsating vacuoles in *Trichomonas hominis* and *T. flagelliphora*, but this is undoubtedly incorrect. No fat globules have been demonstrated.

Frontal and paracostal granules are unusual in Trichomonadinae from termites. The latter are reported to occur in *Ditrichomonas termitis*, at times, and in *Trichomonas trypanoides*. Some granules are sometimes arranged in a line under the costa of *Trichomonas termopsidis*, but they are not conspicuous. Small granules scattered in the cytoplasm are present in these flagellates and frequently they are most numerous near the nucleus. In *Pseudotrypanosoma giganteum* there is an unusually large number of these in the region between the costa and the axostyle and in the anterior region of the body.

In those flagellates which feed on wood it is easy to distinguish between the alimentary vacuoles, containing wood, and the metaplasmic spherules. Of these spherules, some stain with neutral red and some do not, and there are similar differences in stainability with other vital dyes. These all stain black with iron haematoxylin.

The small, spherical, or rod-formed granules which Hogue (1922) and Grassé (1926) stained with Janus green are doubtless mitochondria. Alexeieff (1928, etc.) classes practically all the organelles and permanent granules of trichomonad flagellates as mitochondrial formations. It seems that this conception of mitochondria is overdrawn; though perhaps, as he points out, the action of acetic and the very limited staining capacity of a structure should not be regarded as the final criterion of its mitochondrial nature. Parabasal bodies differ in their staining capacity and apparently chromatin is not always stained by the Feulgen reaction.

PARADESMOSE AND CENTROSOME

The paradesmose is of universal occurrence during division in the Trichomonadidae. It varies in its degree of development in this group from a slender strand to a stout bar, but it is generally conspicuous. Its degree of development is a constant character in each species. Unusual paradesmoses occur in *Pentatrichomonoides scroa*, in which the structure is band-formed, and in *Pseudotrypanosoma giganteum*, in which it is broad and fibrillar. In the latter large flagellate the paradesmose is similar to that of some of the hypermastigotes, notably *Staurojoenina assimilis*. This structure stains well with Heidenhain's iron haematoxylin, but does not stain well with Delafield's haematoxylin.

In some species of Trichomonadinae and *Eutrichomastix,* the paradesmose has been described as connecting the blepharoplasts, which usually come to lie at the poles of the dividing nucleus. A modification of this arrangement is shown by Cleveland (1928) in *Tritrichomonas fecalis.* In this species the paradesmose connects the two blepharoplasts and a rhizoplast extends from each blepharoplast to the nuclear membrane. In *Tritrichomonas augusta* the paradesmose connects the blepharoplasts and there is a separate centrosome. In *Trichomonas linearis* and *P. scroa,* the paradesmose appears to connect the blepharoplasts and there is no separate centrosome.

In a number of Trichomonadinae it has been found that the ends of the paradesmose are separate from the blepharoplast and attached to it by a slender rhizoplast. In some cases there is a distinct granule at the end of this paradesmose and this is called the centrosome. This is the arrangement in *Trichomonas buccalis* (Hinshaw, 1926), *Tritrichomonas batrachorum* (Grassé, 1926), *Ditrichomonas termitis* (Cutler, 1919), *Trichomonas termopsidis* (Kofoid and Swezy, 1919; Andrews, 1925). In most of the species described in this paper, the ends of the paradesmose are connected to the blepharoplasts by slender filaments, and since in the resting stage a rhizoplast extends to the nuclear membrane but does not end in a distinct centrosome, it is probable that the centrosome separated from the blepharoplast at the onset of mitosis. *Trichomonas barbouri, T. cartogoensis,* and *Tritrichomonas brevicollis* show this arrangement. In *Pseudotrypanosoma giganteum* the ends of the broad, fibrillar paradesmose attach to bands which extend to the blepharoplast, but as in *Staurojoenina assimilis,* there is no morphologically distinct centrosome. In the latter species, astral rays radiate into the cytoplasm from the ends of the paradesmose, so that there is evidence of a centrosomal function.

Evidently this separation of centrosome and blepharoplast at the time of division is characteristic of a number of trichomonads and perhaps further investigtaion will show that it is even more general. Therefore, it cannot be used as a basis for the establishment of subgenera, as was done by Kofoid and Swezy (1919) for *Trichomitopsis* and by Andrews (1925) for *Trichomonopsis.*

ORIGIN OF NEW ORGANELLES

The question of the origin of new organelles in flagellates has given rise to much discussion. Now it appears to be well established for most organelles that differentiation of the new structure occurs. The old structure is sometimes resorbed and two new ones differentiated, as in the case of the axostyle of Trichomonadidae, Pyrsonymphidae, and Oxymonadidae; the staborgan of *Peranema* (Brown, 1930) and *Heteronema* (Rhodes, 1926); the entire flagella, axostylar, and parabasal apparatus of *Lophomonas;* the flagellar apparatus and cytoendoskeleton of *Kofoidia* (Light, 1927); and the flagella, blepharoplasts, axostyle, and fibrils of *Proboscidiella* (Kirby, 1928) and *Oxymonas* (Connell, 1930).

In other cases the parent structures are distributed between the daughter individuals and new ones are differentiated to complete the full number in each. This is the case with the costa, and it appears to be true in most cases of flagella. In the Trichomonadidae the flagella appear to be distributed and new ones grow to complete the number, the outgrowth generally being completed before division of the body. In the Calonymphidae, according to Janicki (1915), the parent parabasal body persists in one daughter mastigont and a new one develops in the other. Kuczynski (1914) states that the parabasal body behaves in this way in *Trichomonas,* and Wenrich (1921) suggests that this may be the method of origin in *T. muris.*

The idea of the origin of new organelles by splitting of the parent structure has been suggested and then abandoned for the flagella, costa, and axostyle. C. T. Reed (1930) reports that the flagellum splits in *Peranema globiferum,* but outgrowth of the new flagellum has been shown clearly in *Peranema* by Hall and Powell (1928) and by Brown (1930), and observed by the writer in the closely related *Heteronema acus.* The blepharoplast, and sometimes the rhizoplast, are supposed to multiply by splitting, as does the so-called parabasal body in the Trypanosomidae. Division has been accepted by Duboscq and Grassé (1927) and Grassé (1926) as the method of duplication of the parabasal body.

Alexeieff formerly (1917*b*) believed in the autonomous multiplication of the parabasal body by division, but later (1924) states that resorption of the old and differentiation of the two new structures occurs in *Tritrichomonas augusta.* It has been demonstrated that this is the method of origin of new parabasal bodies in *Trichomonas termopsidis* and it seems likely that it will be found that this method is general among trichomonad flagellates.

SUMMARY

1. *Eutrichomastix axostylis* sp. nov. is described from one of the Termitidae, *Nasutitermes* (*Subulitermes*) *kirbyi.*

2. The new subfamilies Polymastiginae, Devescovininae, and Trichomonadinae of the family Trichomonadidae Wenyon are proposed.

3. The ten previously described Trichomonadinae from termites are reviewed, and seven new species, including one new genus of this family are described from termites. See diagnoses on pages 214 to 219.

4. *Trichomonas termopsidis* Cleveland is reported from *Termopsis laticeps,* in addition to *Termopsis angusticollis* and *T. nevadensis.*

5. New observations on the structure and reproduction of the parabasal body are reported. A series of granules, staining with Delafield's haematoxylin, is found in the parabasal body of *T. termopsidis* and other species. It is shown that in *T. termopsidis* the old parabasal is resorbed and two new ones differentiated in the prophase of mitosis. It is suggested that this is the general method of origin of the structure in Trichomonadidae.

6. A small parablepharoplast bar, from which a filament leads to the nucleus, is found in *Trichomonas barbouri* sp. nov. The bar has been found also in *T. cartagoensis* sp. nov. and *Tritrichomonas augusta.* This structure has not been described before in trichomonads.

7. Two species of *Trichomonas* are described from Termitidae, a family of termites in which Protozoa are infrequent. These are: *T. linearis* sp. nov., which is the only protozoan found in *Orthognathotermes wheeleri;* and *T. labelli* sp. nov., which occurs together with large amoebae in *Mirotermes hispaniolae.* In the former species the body is always straight, not being bent at times as in many other species; in the latter species there is a lip-like projection at the posterior border of the cytostome in some individuals, as described in *T. termitidis* Dogiel and some other species.

8. Two species of *Tritrichomonas, T. brevicollis* and *T. holmgreni,* are described. These have slender, short parabasal bodies.

9. The new genus *Pentatrichomonoides* is established for *P. scroa,* a rather large form with five anterior flagella. This species occurs

in both *K.* (*Cryptotermes*) *dudleyi* and *K.* (*Lobitermes*) *longicollis*, in which termites the remaining faunas differ greatly.

It has an exceedingly slender, quite rudimentary axostyle, the costa is unusually slender for a form of such size, and there is an unusual ellipsoidal parabasal body containing a large granule of Delafield staining substance. An innominate filament, passing from the blepharoplast to back of the nucleus, is described. This is a new structure in trichomonads. A band-formed paradesmose is present in *P. scroa.*

10. *Pseudotrypanosoma giganteum* is reported from *Porotermes grandis*, in which the flagellate fauna is similar to that reported from *Porotermes adamsoni* by Grassi.

Three anterior flagella, a large blepharoplast, an axostyle and possibly a long parabasal body are described in this species. A broad fibrillar paradesmose, like that of some of the hypermastigotes, is present during division.

A small trichomonad with three anterior flagella and a stout axostyle is also present in *Porotermes grandis.* This may be a form of the other species; possibly it is a different species.

11. It is pointed out that size unusual among the Trichomonadinae occurs in only four of the fifteen or sixteen species reported from termites.

12. The contraction and bending of the body in trichomonad flagellates is discussed. It is suggested that this is chiefly a cytoplasmic activity, possibly aided by myonemes, at least in *P. giganteum.* In this connection the question of the activity of the axostyle is discussed.

13. In all the species described in this paper the blepharoplast is a single mass, sometimes flattened on one side or crescentic, and in some it is composed of several granules imbedded in a common matrix.

A rhizoplast occurs in several species, but has not been found in all. A centrosome at the nuclead end of this has not been observed in resting stages.

14. A broad capitulum and posterior enlargement of the axostyle occurs in *T. barbouri* and *T. cartagoensis*, in addition to *T. termopsidis.* The existence of a terminal filament on the axostyle is found to be quite general, and it is suggested that this may occur in other species in which it has not been described.

15. *Trichomastix salina* Entz has been mentioned (Grassé, 1926) as a free living flagellate with an axostyle. It has been found that this flagellate is not a *Trichomastix* or *Eutrichomastix*, and does not have an axostyle.

16. There are two general types of nuclei in Trichomonadinae; one in which the nucleus is entirely or almost filled with granules of chromatin, and one in which the structure is somewhat like that of *Entamoeba*, with a small central mass, peripheral chromatin and sometimes apparently spoke radii. In some cases, the two types have been found in one species.

17. In some species the paradesmose connects the blepharoplasts directly; in some the ends of this are connected by filaments to the blepharoplasts. The latter arrangement occurs in several species described in this paper. It is not an adequate basis for subgeneric distinction.

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EXPLANATION OF PLATES

All figures drawn with the aid of the camera lucida except 81. Abbreviations for methods of preparation: B., Bouin's fluid; Fl., Flemming's fluid without acetic acid; O., osmic vapor; S., Schaudinn's fluid; D., Delafield's haematoxylin; H., Heidenhain's haematoxylin; E., eosin; Er., erythrosin; F., acid fuchsin.

PLATE 14

Figs. 1–5. *Eutrichomastix axostylis* sp. nov. from *Nasutitermes* (*Subulitermes*) *kirbyi.*

Fig. 1. Axostyle, blepharoplast, nucleus. S. H. × 1830.

Fig. 2. Enlarged posterior end of axostyle, periaxostylar rings on projecting portion. S. H. × 2725.

Fig. 3. Cytostome, nucleus, blepharoplast. S. H. × 2725.

Figs. 4–5. Typical forms; axostyles, periaxostylar rings. S. H. × 1830.

Figs. 6–11. *Trichomonas termopsidis* Cleveland.

Fig. 6. From *Termopsis laticeps.* Parabasal body shows faintly. Note form of capitulum and bend in axostyle. S. H. F. × 1375.

Figs. 7–11. From *Termopsis angusticollis.*

Fig. 7. Rhizoplast, anterior portion of capitulum. S. H. × 1335.

Fig. 8. Capitulum with endoaxostylar granules; parabasal body. O. H. × 1335.

Fig. 9. Old axostyle displaced; paradesmose and rhizoplasts; two new parabasal bodies and portion of old parabasal. O. H. × 1335.

Fig. 10. Blepharoplast; axostyle with capitulum, bend, and endoaxostylar granules; parabasal body and parabasal thread. O. H. × 1335.

Fig. 11. Two new axostyles with capitula, posterior enlargements and terminal filaments; two well developed new parabasals; two slender costas in addition to the larger two; both nuclei displaced. Plasmotomy delayed. O. H. × 1335.

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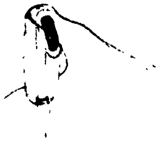
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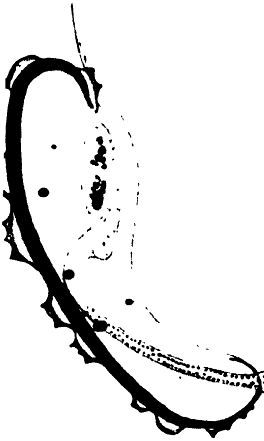
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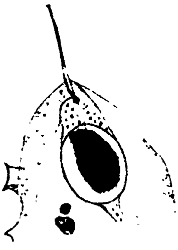
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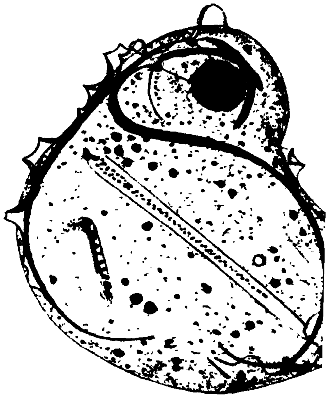
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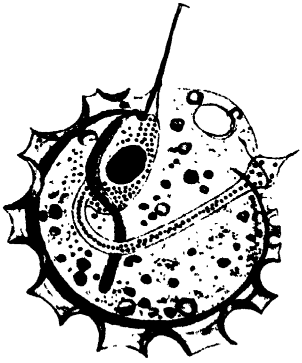
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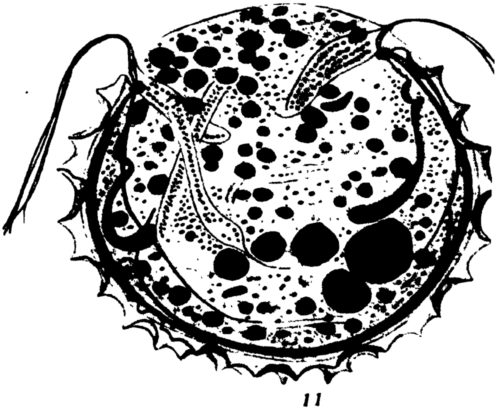
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PLATE 15

Trichomonas termopsidis Cleveland

Figs. 12–16. From *Termopsis laticeps,* showing development of two new parabasal bodies and resorption of old one, as well as the structure of the parabasal body as demonstrated by staining with Delafield's haematoxylin. S. D. F. × 1335.

Figs. 12–13. Prophase; old parabasal dislodged; new parabasals short, slender, and pointed posteriorly.

Fig. 14. Anaphase; old parabasal present, apparently diminished in size.

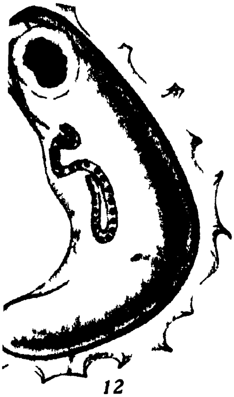
Fig. 15. Similar; old parabasal being resorbed; new ones longer and pointed; abnormal axostyle, apparently degenerating.

Fig. 16. Late anaphase; old parabasal has been resorbed; new parabasals almost full size with rounded posterior ends.

Figs. 17–18. From *Termopsis angusticollis.* Drawn from living, unstained specimens. × 970.

Fig. 17. Typical; projecting axostyle; free flagellum at end of undulating membrane; wood, spherules, and granules in body.

Fig. 18. Dividing animal; two sets of four flagella each; wood, spherules, and granules in cytoplasm.



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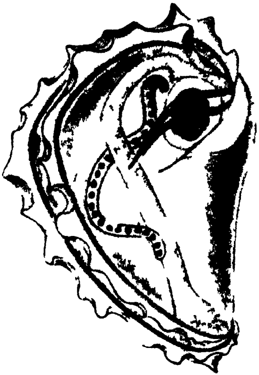

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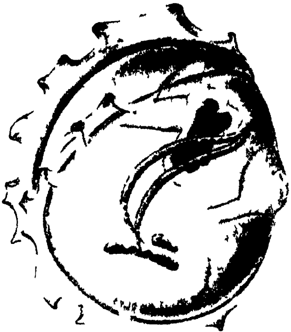

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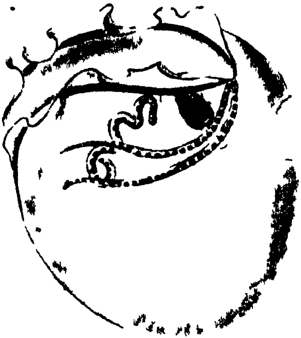

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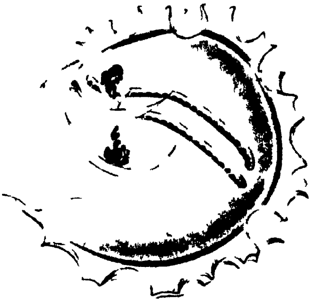

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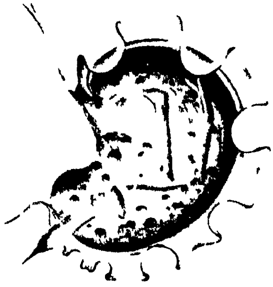

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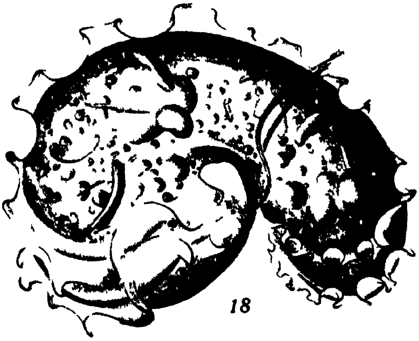

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PLATE 16

Trichomonas termopsidis Cleveland from *Termopsis angusticollis.* × 1500.

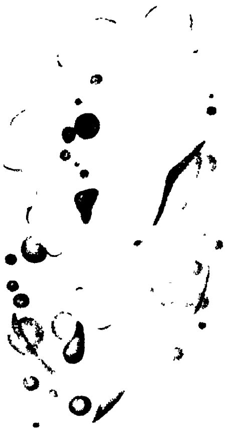
Fig. 19. Living flagellate stained with neutral red.

Fig. 20. Living flagellate stained with brilliant cresyl blue.

Figs. 21–22. From material fixed in Zenker's and stained with Mallory's triple stain.

Fig. 21. The endoaxostylar granules, not shown here, are red; sheath of axostyle blue.

Fig. 22. Numerous red and some purple spherules.



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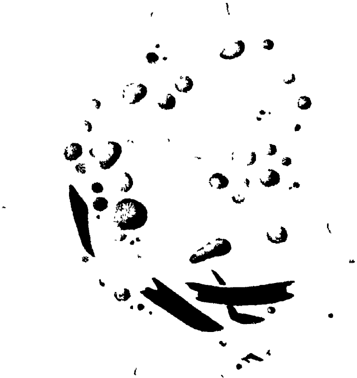

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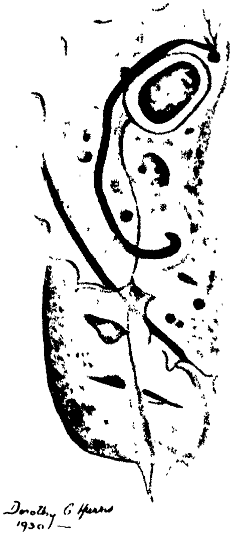

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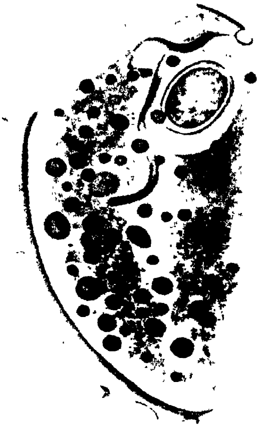

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PLATE 17

Trichomonas barbouri sp. nov. from *Kalotermes* (*Glyptotermes*) *barbouri*.

Fig. 23. Typical; note form of axostyle and parabasal body. Fl. H. F. × 1830.

Fig. 24. Blepharoplast, rhizoplast, parablepharoplast bar with anterior and posterior filaments, parabasal body with filament, nuclear structure. S. H. × 2725.

Fig. 25. Blepharoplast, parablepharoplast bar and its posterior filament extending along nuclear membrane. S. H. × 2725.

Fig. 26. Granular composition of blepharoplast; parablepharoplast bar. S. H. × 2725.

Fig. 27. Beginning of new costa. B. H. E. × 1370.

Fig. 28. Rhizoplast and blepharoplast. S. H. × 1370.

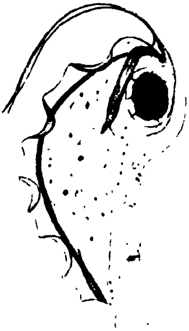
Fig. 29. Parabasal body. Fl. H. F. × 1830.

Fig. 30. Growth of new costa. S. H. × 1370.

Fig. 31. Division; paradesmose and rhizoplasts. S. H. × 1370.

Fig. 32. Late division; paradesmose, one new axostyle, other new one not seen. S. H. × 1370.

Fig. 33. Large, typical form; parabasal; large, reticular nucleus, typical form of axostyle; flagella exact length. Fl. H. F. × 1830.



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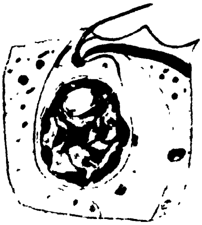

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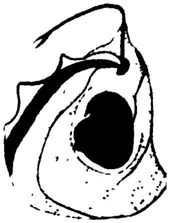

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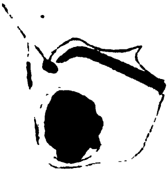

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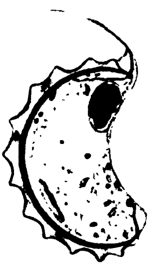

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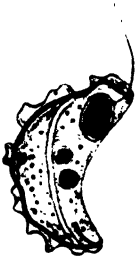

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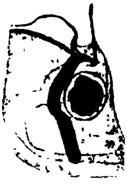

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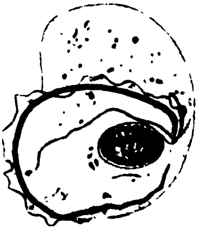

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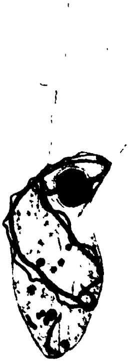

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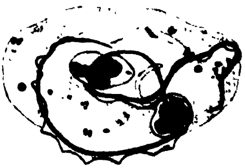

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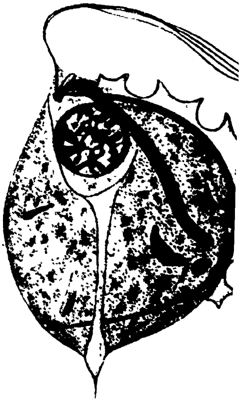

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PLATE 18

Figs. 34–39. *Trichomonas cartagoensis* sp. nov. from *Kalotermes contracticornis.*

Fig. 34. Axostyle and its capitulum; parabasal body. S. D. F. × 1370.

Fig. 35. Cytostome. S. H. F. × 1370.

Fig. 36. Typical form; parabasal body, axostyle. S. D. F. × 1370.

Fig. 37. Early anaphase. S. H. F. × 1370.

Figs. 38–39. Later anaphases. S. H. × 1370.

Figs. 40–45. *Trichomonas linearis* sp. nov. from *Orthognathotermes wheeleri.*

Fig. 40. Blepharoplast, nucleus. S. H. × 2725.

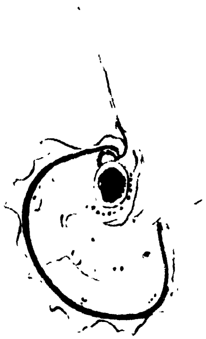
Fig. 41. Typical form; good for axostyle. S. H. F. × 1830.

Fig. 42. Unusually long projecting portion of axostyle. S. H. × 1830.

Fig. 43. Unusually large nucleus; blepharoplast showing granular composition; chromatic blocks and granules in cytoplasm. S. H. × 2725.

Fig. 44. Cytostome; axostyle. S. H. × 2725.

Fig. 45. Late anaphase, showing paradesmose. S. H. × 1830.



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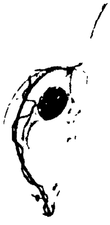

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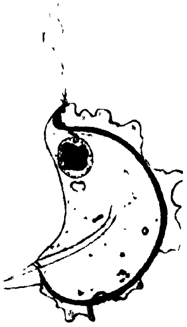

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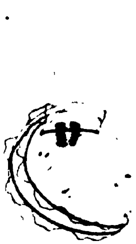

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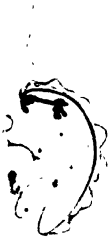

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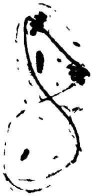

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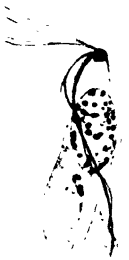

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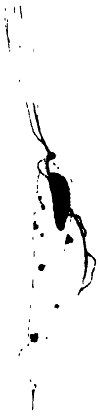

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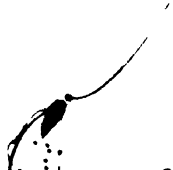

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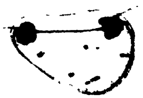

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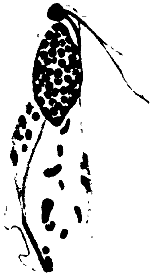

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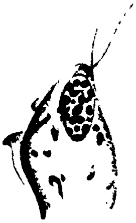

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PLATE 19

Figs. 46–49. ***Trichomonas labelli*** **sp. nov. from** ***Mirotermes hispaniolae.***

Fig. 46. Typical form. S. H. × 1830.

Fig. 47. Projecting lip. S. D. × 1830.

Figs. 48–49. Rounded forms with long projecting axostyles. S. H. × 1830.

Figs. 50–58. ***Tritrichomonas brevicollis*** **sp. nov. from** ***Kalotermes (Calcaritermes) brevicollis.***

Fig. 50. Cytostome, nucleus, axostyle. S. H. × 1830.

Fig. 51. Small individual. S. H. F. × 1370.

Figs. 52–53. Typical forms. Fl. H. F. × 1830.

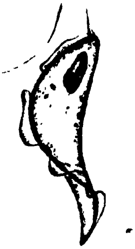
Fig. 54. Diagram, showing enlarged end of paradesmose and rhizoplast connecting to blepharoplast. S. H.

Fig. 55. Rhizoplast, nucleus. S. H. F. × 2725.

Fig. 56. Parabasal body. Fl. H. F. × 1830.

Fig. 57. Anaphase; paradesmose, two new axostyles, discarded old axostyle. S. H. ×1830.

Fig. 58. Telophase, showing paradesmose enlarged at ends from which filaments extend to blepharoplasts. Compare figure 54. × 1830.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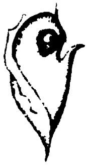
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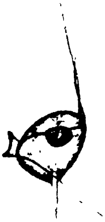
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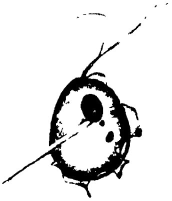
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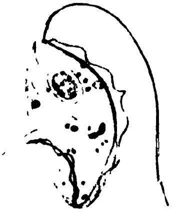
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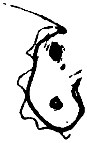
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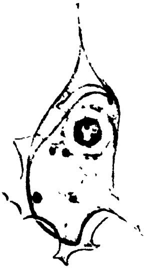
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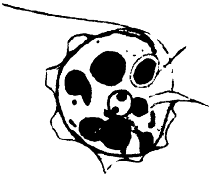
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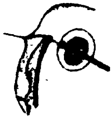
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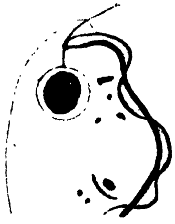
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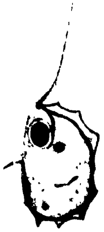
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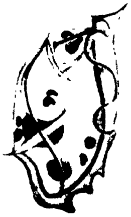
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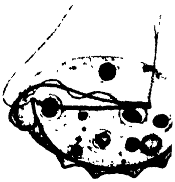
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PLATE 20

Figs. 59–64. *Tritrichomonas holmgreni* sp. nov. from *Kalotermes* (*Neotermes*) *holmgreni*.

Fig. 59. Typical form; nucleus, rhizoplast, parabasal body, projecting axostyle. S. H. × 1830.

Fig. 60. Parabasal body. Fl. H. × 1370.

Fig. 61. Spiral form, trace of parabasal. Fl. H. × 1830.

Fig. 62. Blepharoplast, parabasal, rhizoplast. Fl. H. × 1830.

Fig. 63. Cytostome, parabasal. Fl. H. × 1370.

Fig. 64. Small individual. Fl. H. × 1370.

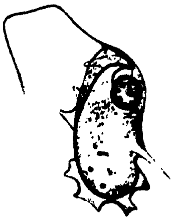
Figs. 65–70. *Pentatrichomonoides scroa* gen. nov., sp. nov. from *Kalotermes* (*Cryptotermes*) *dudleyi*.

Fig. 65. Rounded out body, parabasal. S. H. E. × 1370.

Fig. 66. Scroll-form body, cone at base of flagella, parabasal over which is innominate filament. Fl. H. E. × 1335.

Figs. 67–68. Views of scroll forms from different aspects. Fl. H. E. × 1370.

Figs. 69–70. Blepharoplast, parabasal body, cytoplasmic inclusions. O. H. × 1335.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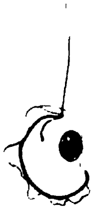
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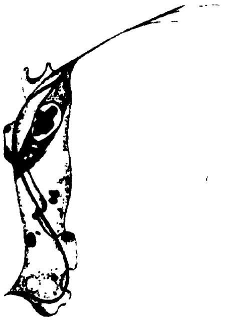
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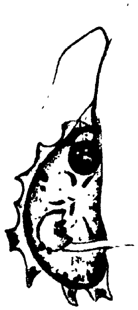
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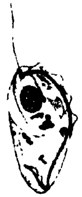
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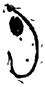
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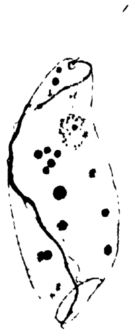
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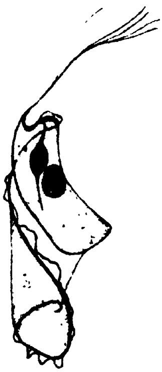
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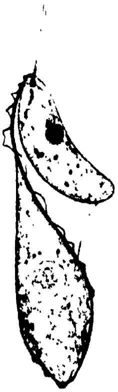
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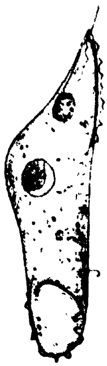
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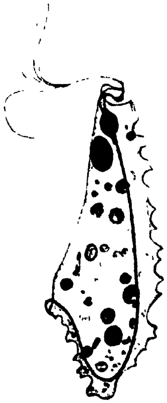
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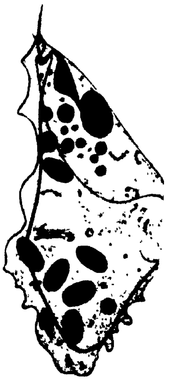
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PLATE 21

Pentatrichomonoides scroa gen. nov., sp. nov.

Figs. 71–84. From *Kalotermes* (*Lobitermes*) *longicollis.*

Fig. 71. Typical scroll form. S. H. × 1830.

Figs. 72–73. Optical section of nuclei showing two inner bodies and chromatin granules. S. H. E. × 1830.

Fig. 74. Surface view of nucleus. S. H. E. × 1830.

Fig. 75. Optical section. S. H. E. × 1830.

Fig. 76. Optical section showing no inner bodies and few chromatin granules. S. D. × 1830.

Fig. 77. Surface view of nucleus with few granules. S. D. × 1830.

Figs. 78–79. Optical sections of nuclei with inner mass surrounded by clear space. S. D. × 1830.

Fig. 80. Two costas and innominate filament; to show that the filament is not a new costa. S. H.·Er. × 1830.

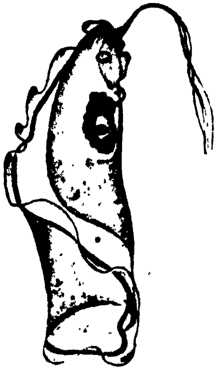
Fig. 81. Enlarged diagram, showing blepharoplast and attachments of organelles; parabasal body; innominate filament and nucleus. S. H. Er. × app. 3500.

Fig. 82. Two costas, innominate filament, and parabasal; to show that the innominate filament is not the parabasal thread. S. H. F. × 1830.

Fig. 83. Broad, band-formed paradesmose. S. H. F. × 1830.

Fig. 84. Telophase; paradesmose. S. H. Er. × 1830.

Fig. 85. From *K.* (*Cryptotermes*) *dudleyi.* Wavy innominate filament. S. H. Er. × 1335.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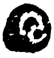
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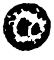
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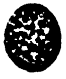
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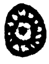
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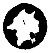
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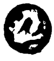
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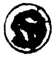
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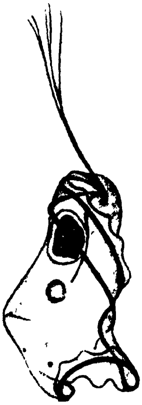
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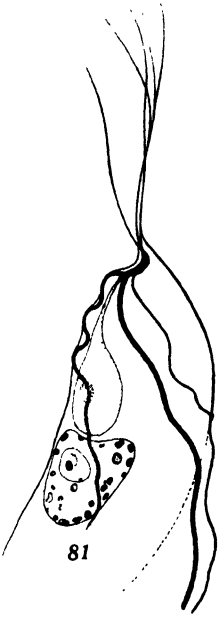
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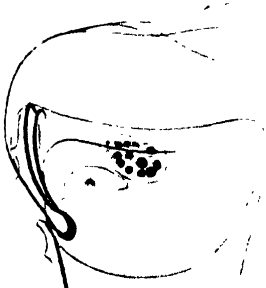
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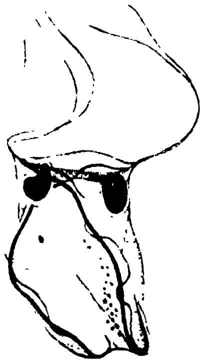
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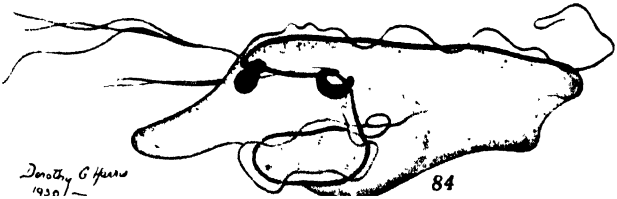
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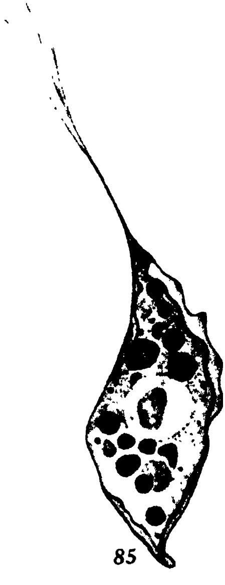
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Dorothy G Hess 1930

PLATE 22

Pseudotrypanosoma giganteum Grassi from *Porotermes grandis.*

Fig. 86. Anterior portion; flagella; blepharoplast; band to nucleus with subtriangular membrane on it near blepharoplast; numerous cytoplasmic granules; axostyle with endoaxostylar granules. S. D. × 1100.

Fig. 87. Typical bent form; axostyle, parabasal body, flagella, blepharoplast not visible. S. H. Er. × 720.

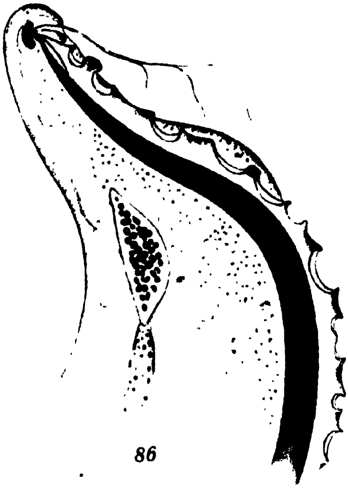
Fig. 88. Striations near surface (myonemes?). S. H. × 720.

Figs. 89–90. Nuclei, showing forms and connections of chromatin granules. S. H. × 1510.

Fig. 91. Anaphase; stout, fibrillar paradesmose, filaments from ends of paradesmose to blepharoplasts, other filaments, chromosomes. S. D. × 1100.

Fig. 92. Anterior portion; blepharoplast, curved lamella anterior to blepharoplast, subtriangular membrane on band to nucleus, new costa, portion of axostyle back of nucleus. S. D. × 1100.

Fig. 93. Smaller trichomonad, systematic status uncertain, from same host. Magnification larger than others, same as isolated nuclei. S. H. Er. × 1510.



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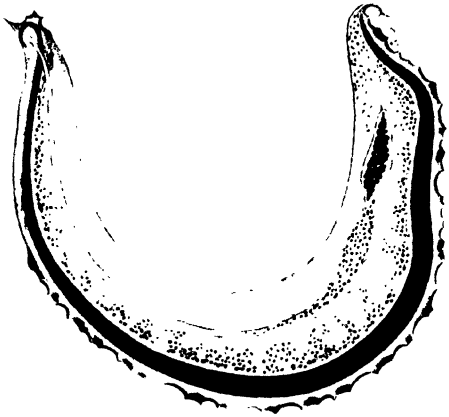

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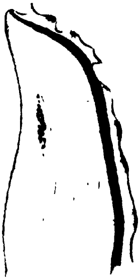

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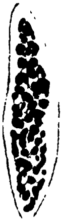

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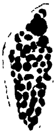

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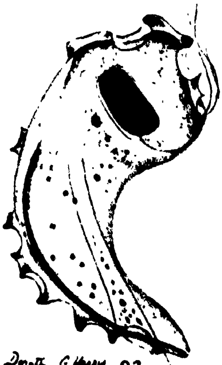

Dorothy G Harris 1930

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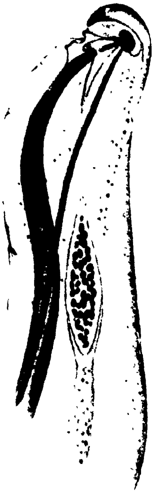

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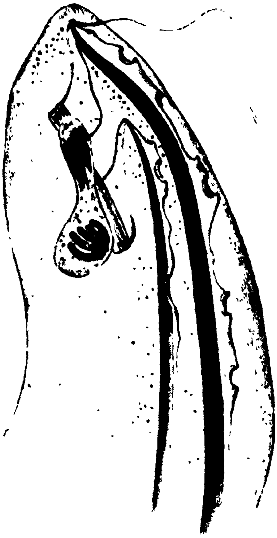

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THE LIFE-CYCLE OF BABESIA BIGEMINA (SMITH AND KILBOURNE) OF TEXAS CATTLE-FEVER IN THE TICK MARGAROPUS ANNULATUS (SAY)

WITH NOTES ON THE EMBRYOLOGY OF MARGAROPUS

BY

EMERY WESTERVELT DENNIS

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THE LIFE-CYCLE OF BABESIA BIGEMINA (SMITH AND KILBOURNE) OF TEXAS CATTLE-FEVER IN THE TICK MARGAROPUS ANNULATUS (SAY)

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INTRODUCTION

The members of the sub-order Piroplasmidea are protozoa that parasitize the red blood corpuscles of mammals, but do not form the pigment that is characteristic of the malarial parasites *Haemoproteus* and *Plasmodium*. They are simple in structure, consisting essentially of a small bit of cytoplasm and a nucleus. The larger forms frequently have a punctiform granule or blepharoplast that is connected to the nucleus by a chromophile fibril or rhizoplast. The piroplasms are typically pear-shaped with the nucleus situated toward the broader end of the parasite (pl. 24, fig. 7).

The sub-order Piroplasmidea is represented by two families, the family Babesiidae Poche (1913) and the family Theileriidae Poche (1913). Members of the family Babesiidae multiply asexually within the erythrocytes of their mammalian host by division into two or more, rarely four daughter individuals, while the members of the family Theileriidae reproduce, also asexually, by a definite process of schizogony within the endothelial cells of the internal organs.

Each family is composed of but a single well defined genus. The genera *Babesia* Starcovici (1893) and *Theileria* Bettencourt, França and Borges (1907) constitute the families Babesiidae and Theileriidae respectively. This paper deals primarily with the largest of the three species of *Babesia* found in cattle, *Babesia bigemina* (Smith and Kilbourne, 1893).

The first observations on the piroplasms were those of Babes (1888) who observed the parasites in the blood of Roumanian cattle which were suffering from haemoglobinuric fever. This observer, however, failed to recognize the protozoan nature of the organism. He named it *Haematococcus bovis,* and believed that he had cultured it *in vitro.* Five years later Smith and Kilbourne (1893) published the results of their epochal work on *Babesia bigemina* in which they not only recognized the true animal affiliations of the parasite, but demonstrated that the disease was transmitted in nature only by the bites of certain ticks. This was the first demonstrated instance of an arthropod serving as a biological vector of protozoan disease.

Smith and Kilbourne named the parasite that they found in the blood of cattle in the southern part of the United States *Pyrosoma bigeminum.* In the same year Starcovici (1893) reviewed Babes' work and named the parasite observed in Europe *Babesia bovis.* He found that *Pyrosoma* had been used as the generic name for an ascidian, and hence was not available, so that Starcovici's name *Babesia* is the valid one. The name *Piroplasma* was suggested by Patton (1895) and has been widely, although incorrectly, used.

The development and life-history of *Babesia bigemina* takes place in two hosts. The parasite reproduces asexually in the red blood cells of the bovine host by binary fission (Smith and Kilbourne, 1893; Nuttall and Graham-Smith [as *B. bovis*], 1908; Dennis, 1930) following a simple sexual cycle in the tick that effects the dissemination of the disease.

Binary fission is inaugurated by duplication of the blepharoplast and rhizoplast which then push out into a pair of symmetrical peripheral lobes of cytoplasm (pl. 24, fig. 8). The lobes grow at the expense of the parent, and fission is completed by division of the nucleus and separation of the two daughters. The two parasites then rupture the host erythrocyte and are free in the blood stream for a short time before entering other corpuscles. The destruction of the red blood corpuscles in large numbers gives rise to the symptoms and pathological manifestations of the disease. In acute cases so much haemoglobin is released by the rupture of the erythrocytes that the urine is red, giving rise to the colloquial name of "red-water fever" for the disease.

It is the purpose of this paper to trace *B. bigemina* through the tick and to describe the various developmental phases. Koch (1906) observed certain "club-shaped bodies" with radiating processes in the gut of ticks which had fed upon blood infected with *B. bigemina.* Dschunkowsky and Luhs (1909) observed similar forms of *B. motasi.* The nature and relationship of most of these forms, however, are still obscure. Crawley (1915) reported finding numerous club-shaped bodies which he described as "gregarinoids" in the ova of *Margaropus annulatus,* which were presumably infected with *B. bigemina.*

The most complete and accurate observations previously published about the development of *Babesia* in the tick are those of Christophers (1907) who made a study of the life-cycle of *B. canis.* Many points in this work were not clear, however, and Christophers' conclusions have not been widely accepted.

Smith and Kilbourne (1893) demonstrated conclusively that *Babesia bigemina* is transmitted from host to host only through the agency of *offspring* of female ticks which had previously fed upon the blood of cattle which, at the time of feeding, were suffering from, or had recovered from an attack of Texas fever. The obligatory vector of this protozoan parasite in North America is the American cattle tick, *Margaropus annulatus* (Say), and the life-history of *Margaropus,* a one-host tick, makes the life-history of *Babesia* inseparable from the developmental cycle of the tick. For this reason it will be necessary to present certain details of its anatomy and embryological development before discussing the developmental phases of *Babesia.*

ACKNOWLEDGMENTS

The writer wishes to acknowledge his deep appreciation of the helpful advice and suggestions of Professor C. A. Kofoid, under whose supervision this investigation was conducted, and his very great indebtedness to Dr. Charles W. Rees, of the Iberia Livestock Experiment Farm, Jeanerette, La., whose kindness and generosity in supplying ticks of demonstrated infectivity and known history made much of the investigation possible. The author also wishes to thank Dr. H. C. Hisel, and Mr. T. W. Cole for their cooperation in securing ticks.

MATERIAL AND METHODS

The ticks used in this investigation were utilized in two ways, namely for a preliminary study of the anatomy, embryology, and life-history of *Margaropus annulatus,* and for studying the life-cycle of *Babesia bigemina* in this tick, its definitive host. The ticks were procured from five sources.

(*a*) Several hundred specimens of *M. annulatus,* including all stages in the life-history of the tick, were procured from Dallas, Texas, through the efforts of Dr. H. C. Hisel, State Veterinarian for Oklahoma. These ticks were removed from cattle which were believed to be carriers of Texas fever and were used in studying the morphology and life-history of the tick.

(*b*) A large collection of *M. annulatus* was very kindly supplied by Mr. T. W. Cole, Inspector in Charge of Tick Eradication, Jacksonville, Florida. A communication from Mr. Cole states:

> These ticks were collected from dairy cattle near Jacksonville, Florida, which we have every reason to believe harbor the micro-organisms of Southern, Splenetic, or Texas Fever.

(*c*) A large number of *M. annulatus* was collected by the writer during the summer of 1929 from dairy cattle just east of Texarkana, Arkansas, where Texas fever was endemic at that time.

(*d*) A fourth supply of fever ticks was collected by the writer in the vicinities of Abbeville and Delcambre, Louisiana, from cattle harboring *Babesia.*

(*e*) The ticks used in tracing the development of *B. bigemina* under controlled conditions were procured through the generosity and kindness of Dr. C. W. Rees, of the Bureau of Animal Industries, United States Department of Agriculture, in charge of the Zoology Division, Iberia Livestock Experiment Farm, Jeanerette, Louisiana.

The development of *Babesia bigemina* as reported here was observed in a series of ticks of known history and demonstrated infectivity. This series will hereafter be referred to as Series A. A second group of ticks, also of known history, but proved to be *non-infective,* were studied to determine normal appearances, and served as controls. The control series will be called Series B.

Series A was obtained in the following manner: A number of adult female *M. annulatus* was removed from a steer that was suffering from an infection of *B. bigemina,* the diagnosis of which was based upon microscopical examination of the steer's blood. It was demonstrated experimentally that these ticks had become infected by placing larvae, which were hatched from eggs laid by some of these females, upon a clean, susceptible bull. This animal reacted positively on the fourteenth day after being infested with the larvae. Both the ova and offspring of these infected females are included in Series A.

Series A was treated as follows:

(*a*) A small incision was made in the posterior lateral margin of the body of an engorged female tick. When such an opening is made in the wall of the body the turgid gut is immediately extruded for one or two millimeters. The exposed gut was washed thoroughly in sterile saline to remove any adhering guanin concretions from ruptured Malpighian tubules. A small incision was then made in the wall of the gut and smears of the exuded contents were prepared on slides or coverslips. This material was studied both in fresh smears in salt solution and in fixed and stained preparations.

(*b*) The incision in the body wall of the tick was extended around the entire body and the dorsal body wall carefully dissected free and turned forward so as to expose the viscera. The gut was then removed and the diverticula fixed for sectioning. The ovary and oviducts also were dissected out and fixed for sectioning. By these methods, both smears and sections of the organs involved in the early history of *Babesia* in the tick were obtained.

(*c*) It was desired to trace *Babesia* through the egg and into the larva of the tick. This was accomplished by isolating infected, gravid, female ticks in large test tubes which contained moist sand, and allowing them to oviposit for three or four days. The eggs were removed from the culture tube each day and placed in labeled tubes to be incubated. Incubation was carried on at 30° C. Some of these eggs were removed at regular intervals during the period of incubation and smears or squash preparations made; at the same time some of these eggs were fixed for sectioning. This method gave a series of preparations showing stages of development from the time of oviposition to the hatching of the hexapod tick larva, thus providing for the correlation of the stage in the cycle of *Babesia* with the stage of development of the tick.

Series B was obtained from female *M. annulatus* which had been reared upon a mule in order to clean them of *Babesia bigemina*. This series was proved to be clean of infection by rearing the ticks for two subsequent generations on susceptible steers without producing any symptoms of Texas fever. Series B was studied in the same manner as Series A in an effort to eliminate parasites not belonging in the life-cycle of *Babesia*. Microscopical examination of these ticks failed to show any *Babesia*.

Dissection.—In studying the anatomy of the adult tick, and in dissecting out organs to be fixed for sectioning, the tick was usually opened by taking it between the thumb and forefinger of one hand and making a lateral incision around the body. The tick was then placed in a shallow vessel containing salt solution and the viscera were exposed by holding the ventral part of the tick to the substrate with a needle and gently pulling the dorsal wall of the body free from the musculature by means of a pair of fine forceps. In some cases it was advantageous to partly imbed the tick in a mixture of paraffin and beeswax before beginning the dissection. Scalpels made of slivers of safety razor blades inserted in the end of a wooden needle handle proved to be very useful. The ticks were usually not killed before dissection.

Squash preparations were made by snipping through the anterior margin of the body wall of the tick, adult or larva, and gently squeezing the body between two slides. The slides were immediately placed in fixing fluid. This type of preparation proved to be much more useful in dealing with the larval stages than the smear, because the destruction of the tissues in it is much less than in the smear.

Fixation and staining.—Bouin's and Schaudinn's fluids, both used after warming to 30° C, were the most satisfactory fixatives. Smears which were to be stained with Giemsa were fixed either in alcoholic sublimate or acetone-free methyl alcohol. The Bouin-chloroform mixture suggested by Wenyon (1926, 2:1326) was useful in the fixation of ticks *in toto*.

Sections of tissues were stained with Heidenhain's iron-alum haematoxylin, Delafield's haematoxylin, or Ehrlich's acid haematoxylin. Erythrosin was used to advantage as a counterstain. Smears were stained with iron-haematoxylin, Delafield's or Giemsa. Giemsa's stain was useful in correlating observations with those of Christophers (1907) and others who used Romanowsky stains almost exclusively.

Clearing and imbedding.—Oil of cedarwood was the most useful medium used for clearing tick tissues. Xylol and chloroform were useful in clearing ova which were to be sectioned. Tick ova were very difficult to section and the results were never very satisfactory, although little difficulty was experienced in sectioning the ova in the ovary and oviduct.

Imbedding proved to be a very important factor in attempts to section ticks and ova. For sectioning the gut and ovary, parawax was a very satisfactory paraffin. For sectioning ticks and ova it was necessary to use paraffin with a melting point of 68–70° C, and to cut the sections at eight microns. Infiltrating *in vacuo,* and then under very slight pressure for a few minutes was helpful in working with nymphs and adult ticks.

THE LIFE-HISTORY OF MAGAROPUS ANNULATUS

The pioneer work upon the life-history and biology of the cattle tick was carried out by Cooper Curtice (1891, 1892), and a fairly complete summary with additional details has been presented by Hunter and Hooker (1907), and by Cotton (1908). The outline here summarized is taken from data given by these authors.

The life-history of *Margaropus annulatus* may be divided into two distinct phases, a parasitic period during which the tick is attached to its host, and a non-parasitic period during which the tick is at no time attached and during which it does not feed. The parasitic period begins with attachment and ends when the mature tick drops from the host and seeks a protected site on the ground for oviposition. After the tick has left its host there is a pre-oviposition period ranging from three days in summer to as many as twenty-eight days in winter. Oviposition usually begins about seventy-two hours after the tick drops from its host, and continues for eight or nine days.

The average number of eggs laid by a single female is about three thousand. The eggs are generally elliptical in shape but may vary because of pressure. The average size of the eggs is about 0.54 by 0.42 mm. (Hunter and Hooker, 1907), and they range in color from a yellowish to a deep brown. The average period of incubation is about thirty days, according to the temperature and the amount of moisture. The seed ticks that emerge from the eggs are minute hexapod creatures which become very active and move up onto a blade of grass or the highest available object, where they bunch in masses of a thousand or more and await the coming of a host animal.

The parasitic period begins when a suitable host animal brushes against the perch of the bunched seed ticks and they transfer themselves to it. If it is a suitable host they will attach themselves and will begin to feed about two days later. The feeding continues for seven to twelve days and the seed ticks molt *on* the host. The result of the first molt is the eight-legged, slaty-gray nymph. The nymphs feed from five to twelve days, then molt once again *on* the host to produce the sexually mature males and females which soon mate and continue to feed on the host from four to fourteen days; when engorged, the adult females drop to the ground. It has been our experience that oviposition begins almost exactly seventy-two hours later. *Margaropus annulatus* is thus capable of completing its life-cycle in about sixty days, making three generations a year possible under the limitations established by temperature.

THE ANATOMY OF THE TICK

The internal morphology of *Margaropus annulatus* has been worked out by Williams (1905) and more completely and accurately by Allen (1905), hence a detailed description would be out of place here. As the alimentary tract, reproductive system, and salivary glands are involved in the life-history of *B. bigemina,* the structure of these organs must be considered in sufficient detail to enable us to correlate the life-cycle of *Babesia* with the organs in which it occurs. As our observations coincide with the descriptions given by Allen, his paper may be referred to for further details.

The *alimentary tract* of the tick is complicated in appearance, but rather simple in structure (fig. A, *1*). It consists of a median canal beginning at the mouth and ending posteriorly in an intestine that is without a lumen, forming a blind sac from which two lateral and one posterior diverticula arise. Beginning with the opening of the mouth, the oesophagus goes almost directly posteriorly, then turns sharply toward the antero-ventral part of the body, only to curve abruptly backward and upward to pass through the brain in about the median line (pl. 23, fig. 6). This course may be more direct in some specimens. After it passes through the brain, the tract extends posteriorly giving off opposed right and left branches. Almost immediately the main line runs ventrally to the cloaca as a blind lumenless intestine; a single branch, the posterior diverticulum, extends posteriorly as originally in the median line. Each of the right and left branches give rise immediately to five finger-like diverticula, the most anterior of which is the longest. The single posterior diverticulum gives rise to

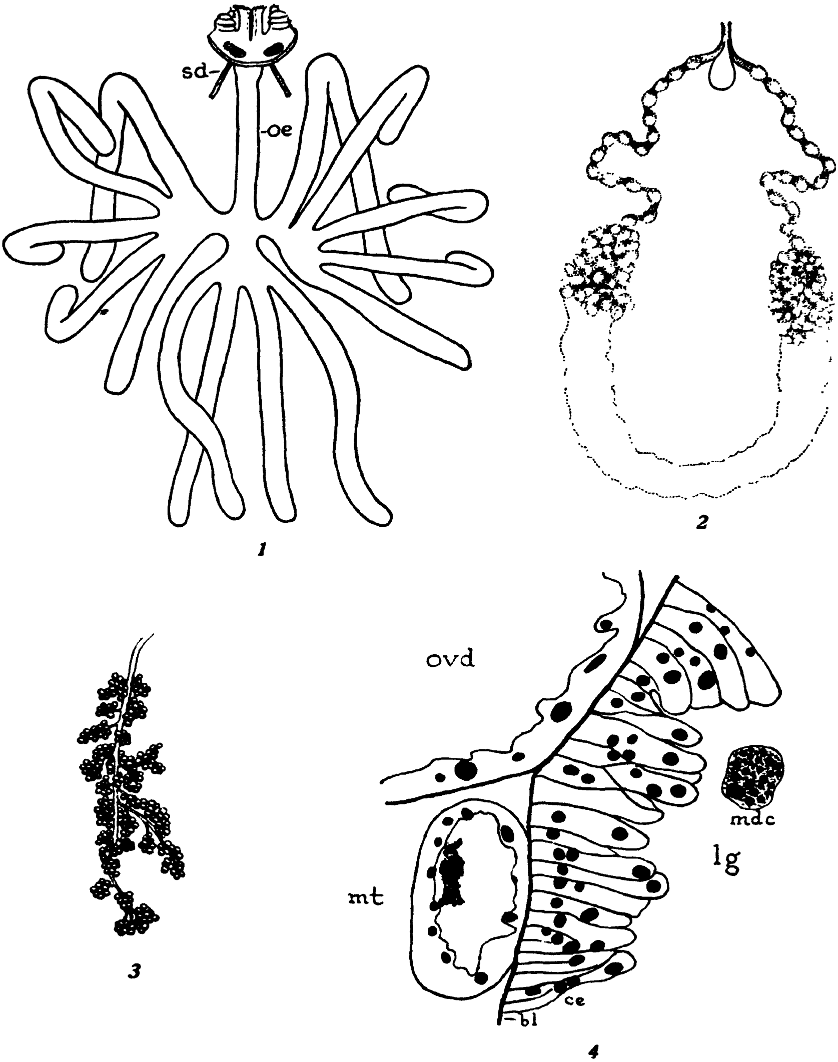

Fig. A. (*1*) A semidiagrammatic drawing of the digestive tract of *Margaropus annulatus*, dorsal view, showing lateral and posterior diverticula, × 5. (*2*) Semidiagrammatic drawing of the reproductive system of a female *M. annulatus*, showing the single ovary connecting the two convoluted oviducts that terminate at the anterior ovipositor. The seminal receptacle is the anterior flask-shaped structure, × 5. (*3*) A single salivary gland, redrawn from Allen, × 20. (*4*) A camera lucida drawing of a portion of a transverse section of an unengorged female *M. annulatus*, showing the nature of the wall of the gut, and the intimate relationship of the gut and the reproductive organs, × 150.

bl., basal layer; *ce.*, columnar epithelium; *lg.*, lumen of gut; *mdc.*, macrophage digestive cell; *mt.*, Malpighian tubule; *oe.*, oesophagus; *ovd.*, oviduct; *sd.*, salivary duct.

four similar branches. Thus there are seven blind branches of the gut on each side of the median line. These caeca are long, extending first peripherally, then ventrally, and finally median again. They branch again in minor secondary pouches due to expansion of the wall of the gut into all available spaces between the muscles and the reproductive system.

In the engorged gravid female the diverticula of the gut and the tubular organs of the viscera are almost inextricably tangled together. This fact is significant because of the large amount of surface of the digestive tract which is brought into intimate contact with the organs of the reproductive system into which it is necessary for *Babesia bigemina* to enter in order that its life-cycle may be successfully completed (see fig. A, *4*).

The histology of the gut is likewise important in its relation to the behavior of *Babesia*. The wall of the gut is composed of two layers, both thin, and when the gut is distended the thickness is so reduced that the passage through it by a parasite could be accomplished with great ease. There is an outer cellular layer, serving as a basement layer, that is rarely over five microns thick and is usually thinner. The other layer is an inner columnar cell layer, composed of somewhat amoeboid, club-shaped cells that extend into the lumen of the gut as single-celled villi, and is about thirty microns in thickness. In the lumen of the gut there are large amoeboid macrophage digestive cells, filled with red blood cells of the bovine host in various stages of digestion, and with dark chromophile masses which resemble the amorphous chromatoidal masses seen in *Endamoeba gingivalis*. The cells of the wall of the gut are secretory and have large granular nuclei. The columnar epithelium is a very loose structure so that the basal layer is essentially the only part of the wall of the gut which a parasite would have to penetrate (fig. A, *4*) in order to enter the reproductive organs.

The *female reproductive system* (fig. A, *2*).—The only external opening of the reproductive system of the female is the anteriorly situated ovipositor. The ovipositor opens internally into a relatively large cavity, the seminal receptacle, into which there also open two long, bilateral, convoluted tubes which are about 17 mm. in length. These tubes, the oviducts, are connected posteriorly by the single convoluted ovary, thus forming a complete loop that is very long. This system is wound in and out among the diverticula of the gut so that some part of it is in close contact with or very close to some branch of the gut at almost all points. The fact that the parasites enter the eggs is apparently not a matter of a tropism or preference; there is essentially no other place to go!

The *salivary glands* (fig. A, *3*) are a pair of compound alveolar glands resembling an elongated cluster of grapes. They are somewhat over two millimeters in length, and in the unfed tick they occupy about a fifth of the volume of the body. The salivary ducts enter the floor of the buccal cavity. The salivary glands also become entangled with the anterior caeca of the gut and the reproductive system.

THE EMBRYOLOGY OF MARGAROPUS

The literature on the embryonic development of ticks is meager and textbooks give no information about the subject. Christophers (1906) in his study of ticks preliminary to his work on the life-cycle of *Babesia canis*, gave a three-page account of the structure of the ovum and the embryology of ticks, but the description is too generalized to be of much use. There are two papers which give adequate accounts of the development of ticks: Wagner (1894) did a splendid piece of pioneer work on *Ixodes calcaratus*, and Bonnet (1907) gave an excellent description of the embryology of *Hyalomma aegyptium*. Apparently the ixodid ticks differ from each other in their development only in minor details, for Bonnet states, in reference to Wagner's paper: "J'ai pu verifier les observations de cet auteur et mes recherches ont confirme entierement ses conclusions." Except for a few minor discrepancies, Bonnet's description holds for *Margaropus annulatus*.

The mature eggs of *M. annulatus* are about 450μ in length, and 420μ in breadth. The ovum consists mostly of spherules of yolk which are imbedded in cytoplasm that appears to be coarsely granular when stained with iron-haematoxylin. The germinal vesicle is located toward the future postero-ventral surface of the egg in an area of the cytosome that is free of yolk (pl. 23, fig. 1).

Fertilization of the egg takes place in the oviduct of the female tick when the egg is freed from its ovarian follicle. Segmentation is usually well under way by the time that oviposition takes place.

Segmentation is of the superficial type, and at first involves only the area of the egg in which the germinal vesicle was situated. As the result of mitotic divisions of nuclei in this region, there is formed a thin germinal disc. This germinal disc, as the blastoderm, extends its margins until the entire yolk mass is enclosed by a cellular layer which is one cell in thickness (pl. 23, fig. 2).

The differentiation of the endoderm begins in the postero-ventral region where segmentation first began. Here proliferation of cells from the blastoderm results in the formation of a loosely aggregated mass of endodermal cells which soon extends anteriorly along the floor of the blastodermic vesicle as a narrow band or ridge of tissue (pl. 23, figs. 2, 3, 4). The process of endoderm formation in the tick was considered as a process of gastrulation by Bonnet, who suggested that the region in which the formation of endoderm begins is comparable with the region of the blastopore in higher forms. In the earlier stages of endoderm formation, nuclei are to be found which have migrated deeper into the yolk mass; these are the endo-vitelline cells of Bonnet. Bonnet described the endoderm as being formed by the migration of these endo-vitelline cells to the periphery, but the writer has seen no evidence that cells migrate deep into the yolk and then *return* to the periphery to form tissue.

As in *Hyalomma,* the formation of mesoderm takes place as a proliferation of the ectental line on both sides of the endodermal ridge. These cells then migrate medially between the ectoderm and the endodermal band, and the mesodermal tissue grows anteriorly and dorsolaterally.

While the endoderm and mesoderm are being defined, proliferation of the ectoderm of the embryonic disc continues until it forms a relatively thick anterior plate that extends to the dorsal surface, and a similar but less extensive plate posteriorly as a caudal lobe (pl. 23, figs. 2, 3). At the same time a thin non-cellular cuticle, which is the outer body covering of the embryo and larva, appears outside of the peripheral blastoderm.

The anlagen of the appendages appear very early as six pairs of lateral buds of mesodermal tissue with a thin ectodermal covering (pl. 23, fig. 4). The two anterior pairs are situated toward the median line and form the mouthparts. The other four pairs are limb buds, but the most posterior pair is quickly resorbed to a small compact mass of tissue that gives rise to the fourth pair of limbs at the first larval ecdysis.

With the appearance of the limb buds, development becomes centered in the anterior end. The greater part of the anterior ectodermal plate forms the large ventral cervical ganglion or brain (pl. 23, figs. 5, 6). This body of cells acquires a lumen in the larger anterior mass, while according to Bonnet the smaller posterior portion which lies in the abdominal region gives rise to six pairs of ganglia.

The development of the endoderm is arrested for a considerable period of time following the completion of the primary endodermal ridge, while the other germ layers continue to develop with rapidity. The Malpighian tubes are about the first endodermal structures to appear. These structures appear as a pair of short tubes, one on each side of the midline in the postero-ventral part of the embryo. They can be seen quite clearly through the shell of the egg, and appear to arise directly from the yolk mass. As the Malpighian tubes grow they become filled with excretory concretions and appear as dark filaments. Short and rod-like at first, these tubes extend posteriorly, then dorsally, and finally anteriorly until each tube is shaped like the letter *U* with the base of the letter directed posteriorly. The excretory vesicle soon appears at the point of origin of the Malpighian tubes as a single chamber into which the tubes empty (pl. 23, fig. 6). An invagination of the ectoderm occurs at this point, and later breaks through forming an excretory orifice.

The buccal apparatus is formed concurrently with the appearance of the Malpighian tubes, from the first two pairs of appendages. The median first pair form the chelicerae, and the second pair form the palpi. The hypostome is formed by a ventral projection of the chitin of the body wall. The hypostome forms the floor of the buccal cavity, while the roof is formed chiefly by the appendages.

The salivary glands are formed postero-lateral and slightly ventral to the buccal apparatus from ectodermal cells of the larger anterior cell mass which are distinguishable by their larger size and the size of the nuclei (pl. 23, fig. 5).

The mesoderm goes chiefly to form the musculature of the tick. The earliest muscles to appear are the dorso-ventral muscles of the posterior region which arise by the muscle fibers penetrating the yolk and growing dorsally until they come in contact with the dorsal wall of the body. Since the endoderm does not appear for some time except in the anterior region, the appearance and arrangement of the muscles delimits the distribution of the endoderm and the pattern of the digestive caeca.

At the time of hatching, the external form and the appendages of the larva have been attained, the nervous system has reached its maximum development, the salivary glands and excretory organs are well developed, but the digestive system consists of little more than the buccal cavity, short pharynx, and the oesophagus, as the postero-dorsal two-thirds of the yolk mass has yet to be enclosed by the endoderm (pl. 23, fig. 6).

BABESIA BIGEMINA IN THE TICK

The sexual phenomena and sporogony of *Babesia bigemina* take place in the tick. The early phases in the developmental cycle occur in the lumen of the gut after ingestion of parasitized blood by the tick. The development of *Babesia* then proceeds particularly in the reproductive system where the ova of the tick are invaded and subsequently in the tissues of the embryonic stages of the tick.

The early stages of development were studied in fresh saline preparations, smears, and sections of the gut, but in no case were the parasites found in the gut in large numbers. Careful examination of the material, however, resulted in the detection of several forms of the parasite.

There were typical trophozoites which had recently been taken into the gut. These parasites were either free in the ingested mass, or incorporated in the wandering digestive macrophages or "liver" cells of the tick. Sometimes several occurred in a single macrophage. Parasites that are so phagocytized soon show signs of being digested. Pear-shaped and rounded forms of the parasite, mostly the latter, were observed (pl. 24, figs. 13 to 18).

In addition to the trophozoites in the gut, there are also present very characteristic forms which correspond to some of the "club-shaped bodies" described by Christophers (1907) as one of the early stages in the development of *Babesia canis* in the tick. These parasites (pl. 24, figs. 21, 25, 26; pl. 25, figs. 30 to 32) are the isogametes. There is also a form that is almost herpetomonad in appearance, but which shows no neuromotor structures, and stains very poorly (pl. 24, figs. 22, 23, 24). These parasites are probably degenerating.

The gametes are about 5.5–6.0μ in length, and have an appearance much like that of the vermicules of *Hepatozoon* in the mite (Miller, 1908) although they are frequently more flattened, or club-shaped. They arise simply by growth and slight modification of structure (pl. 24, figs. 19 and 20) from trophozoites that are indistinguishable from other trophozoites, rather than by the elaborate method described by Christophers (1907) for the origin of the "club-shaped bodies." The gametes could not be identified as to sex, all gametes being identical in appearance. These sexual forms are actively motile: when floating free in a fluid medium they undergo flexure from side to side, the more pointed end or "tail" being the more active: if attached to a substrate they show gregarine-like movement, proceeding with the blunt end foremost. As they progress, small protoplasmic processes best described as "papillae" are thrust out tentatively from the blunt anterior end.

When stained with iron-haematoxylin, the gametes show marked differences from the trophozoite-gametocyte. The cytoplasm is less homogenous, and the nucleus is larger and richer in chromatin. The chromophile extra-nuclear organelles, the blepharoplast and rhizoplast, are typically absent, but sometimes a well defined granule may be seen near the nucleus, and a fibril may be traced for a short distance toward the nucleus (pl. 24, figs. 25 and 26). The blunt anterior end usually stains more intensely than the rest of the cytoplasm.

Fertilization apparently takes place by the isogametes becoming associated first at the anterior end, as in *Hepatozoon* (Miller, 1908) and then fusing (pl. 24, figs. 21 to 24). The actual fusion of the gametes was not observed, and associated pairs are very scarce in the stained preparations; however, paired or associated gametes were observed in fresh smears of the gut contents in salt solution from six of the adult female ticks of Series A (pl. 25, fig. 32). These associated parasites were studied for lengthy periods but no further development was observed before they died or the preparation became too dry. Rounded bodies exhibiting two distinct nuclei (pl. 25, figs. 33 and 34), were present in stained preparations, and have been interpretated as later stages in syngamy.

Syngamy results in the formation of a motile zygote or oökinete which soon leaves the lumen of the gut. The oökinetes (pl. 25, figs. 35, 36, 37, 38, 40, 41, and 42) range from about 7.0 to 12.0 microns in length, and they are more characteristically club-shaped. The oökinetes usually show some structure at the anterior end. In the smaller, and probably younger forms, there is a small papilla-like structure (pl. 25, fig. 35). The larger individuals (pl. 25, figs. 38, 41)) have a cup-like crown which probably functions as a boring organ. This structure is lost shortly after the oökinete enters the egg of the tick (pl. 25, figs. 37, 40, and 42).

The oökinetes pass through the wall of the gut of the tick and into the contiguous organs of reproduction where they invade the ova. Usually only one or two oökinetes enter a single egg, but approximately fifty were observed in one instance. In our experience only the larger ova are entered.

In the ovum the oökinete forms a *sporont* (pl. 25, fig. 43). The sporont has a large vesicular nucleus, and forms a cyst wall about itself. The smallest sporont observed measured 7.5μ in diameter, and the largest one measured 12.0μ in diameter, exclusive of the wall of the cyst. The nucleus of the sporont or zygote divides (pl. 25, fig. 44) and there are formed within the cyst a number of *sporoblasts* (pl. 26, figs. 45 and 46).

The sporoblasts are somewhat amoeboid, many of them exhibiting fine radiating pseudopodia. The nuclei of the sporoblasts increase in numbers to form multinucleate amoeboid somatellas which migrate throughout the embryonic tissues of the developing tick. Because of their migratory activities we call the parasites *sporokinetes* (pl. 26, fig. 47) following the usage of Reichenow, 1921.

Owing to their amoeboid nature the sporokinetes are very pleomorphic. They may be round, asteroid, club-shaped, or drawn out into long tenuous forms as much as 15μ in length. The nuclei appear as well defined granules about 0.4μ in diameter, and range in number from four to about thirty-two. During the ontogeny of the tick the sporokinetes apparently form other sporokinetes by simple plasmotomy. Practically any tissue of the embryo of the tick may be infected. In many cases the cytoplasm of the host cell may be almost completely displaced by the parasites. Since much of the anterior embryonic cell mass is destined to become salivary tissue, some of the alveoli of the salivary glands come to be occupied by sporokinetes. As the period of incubation of the tick comes to a close, some of the sporokinetes undergo sporogony to form *sporozoites;* others may not form sporozoites until after the tick has hatched, as sporokinetes have been observed in the tissues of active larvae.

The sporozoites (pl. 26, fig. 49) are miniature trophozoites. They tend to be pyriform, have a characteristic nucleus, and the extranuclear granule or blepharoplast soon appears. The sporozoites are most numerous in the salivary glands and the connective tissue at the base of the limbs and surrounding the organs of the viscera. In some cases the contour of these small parasites gives the impression that they are dividing in much the same manner as does the trophozoite. The sporozoites are inoculated into the blood stream of the bovine host in the saliva of the feeding tick larva, and there set up an active infection of the red blood cells.

DISCUSSION

The only previous attempts to make a systematic study of the life-cycle of *Babesia* in the tick were those of Koch (1906) and Christophers (1907). Practically all other contributions to our knowledge of the subject have been random observations which were but incidental to the subject under consideration. The paucity of trustworthy observations is largely due to the fact that the economic importance of babesioses has centered interest upon biológical control of *Babesia* by the eradication of the vector involved. Furthermore, the bewildering display of débris presented by a smear of the contents of the gut of a tick would immediately discourage anything less than a thoroughly conscientious attempt to find parasites therein. Also, when one considers that there are relatively few *developmental* forms of *Babesia* in the gut of the tick at any time and that the multiplication period of the parasite in the tick does not occur until near the close of its sexual cycle, it becomes more clear why the phases of development in the gut have been overlooked.

There is general agreement in the observations of Koch (1906), Christophers (1907), and those recorded above on the forms of *Babesia* present in the gut, but considerable discrepancy in interpretations. No investigator has been able to distinguish gametocytes from normal trophozoites of *Babesia*, and a false analogy of the life-cycle of *Babesia* with that of the malarial parasites has led to confusion.

Since nothing identifiable as a gametocyte has been found, it is agreed that the developmental forms of *Babesia* in the tick must arise from normal-appearing parasites which are freed in the gut. The isogametes arising from such normal-appearing forms were seen by both Koch and Christophers.

Koch (1906) observed that in the gut of *Rhipicephalus australis*, *R. evertsi*, and *Hyalomma aegyptium*, *B. bigemina* increased in size and produced long radiating pseudopodia or processes. These "Stechapfelformen" were observed associated in pairs, a phenomenon which Koch suggested was a kind of conjugation. The ray-like processes of the associated individuals were retracted and the two fused to form a globular body with two nuclei (*see* Koch, 1906, pl. 2, fig. 24). This globular form is undoubtedly the same as the one observed by us (pl. 25, figs. 33 and 34).

Christophers (1906*a*), in a preliminary note on the life-cycle of *B. canis,* reported a pairing of "club-shaped bodies" which gave rise to a rounded parasite exhibiting two nuclei and an achromatic line indicating the line of fusion. In his subsequent paper, however, Christophers (1907) reversed his conclusions, describing the formation of the club-shaped bodies by a very peculiar method of division of the larger rounded parasite. He does not state his reasons for the change in his interpretation, but the final paragraph of his description of the formation of the "club-shaped bodies" indicates that it was because he did not find the thing for which he was looking. Christophers (1907, p. 59) states:

> The method of development in the tick certainly suggests a sexual cycle, and it is immediately prior to the formation of the club-shaped bodies that one would expect the junction of the male and female elements to occur. As just stated, the double forms are not conjugation forms and the analogy of the development of piroplasma with that of the malarial parasite would lead one to suppose that the male element is small, and in such a situation as the gut of the tick likely to be easily overlooked. The absence of any definite observation regarding fertilization is not therefore sufficient at this stage to negative the view that the life cycle of development in the tick is a sexual one.

The only essential difference in the observations of Koch, Christophers, and the writer on the early forms of *Babesia* in the gut is relative to the ray-like processes of the "Stechapfelformen" described by Koch. Kleine (1906), Nuttall and Graham-Smith (1908*a*), Martini (1909), Knuth and Richters (1909), Thompson and Fantham (1913), and Ziemann (1913) have observed the formation of radiating pseudopodia in so-called "culture" forms of *Babesia.* The writer, however, is in agreement with Christophers (1907) in concluding that the formation of these ray-like processes is not essential in the development of *Babesia.* We have seen the production of very similar processes by *Trichomonas* from old cultures and in fresh smears which had become too dry, so that it seems probable that such processes are induced in *Babesia* by unfavorable culture media or by drying.

In previous work, the "club-shaped bodies" have dominated the life-cycle of *Babesia.* Koch (1906) saw certain club-shaped bodies in the ova of the tick, which were about four times the size of the trophozoites. Christophers observed club-shaped bodies in the gut, in the tissues of nymphs, and in the reproductive organs of adult female ticks. No distinction was made between the gametes and the oökinete, but Christophers did describe the formation of "zygotes" by the rounding up and growth of club-shaped bodies. In view of the fact that the so-called "club-shape" is characteristic of the oökinetes of the Haemosporidia, and that *B. bigemina* assumes a "club shape" several times in its life-cycle, the club shape should be considered as a consequence of the method of locomotion and tissue-penetrating activities of the micro-organism and has no special morphological significance as a "stage" in its sexual cycle.

The zygote of *B. bigemina* is present in three forms. It appears as the rounded body formed as the result of syngamy, as the active oökinete, and as the sporont in the ovum of the tick. Christophers described a "zygote" which was imbedded in the tissues of the gut and in the yolk of the egg. This "zygote" is considered to be homologous with the sporont of *B. bigemina*, although according to Christophers a cyst wall was never formed. The wall of the cyst of the sporont of *B. bigemina* was difficult to define in some cases, being indicated only by a sharp halo around the sporont, but it appeared distinctly in sectioned material stained with iron-haematoxylin (pl. 25, figs. 43 and 44). In some cases Christophers may have been dealing with young sporonts which had not yet produced the cyst, but his observations were made mostly on squash preparations stained with Giemsa and many of the "zygotes" figured by him look more like tissue cells than sporonts. Since there is no reason to expect marked differences between the individuals of *B. canis* and *B. bigemina* in corresponding stages of their life-cycle, it seems probable that some of the "zygotes" that Christophers was dealing with were artifacts.

The sporoblasts are but transitory forms in the life-cycle of *B. bigemina.* There is nothing outstanding about them except that they eventually give rise to the active sporokinetes which are responsible for the spreading of the parasite throughout the embryonic tissues of the tick. The formation of sporoblasts in the life-cycle of *B. canis* was described by Christophers, who included forms which were probably sporokinetes.

The sporokinetes are particularly important in the life-cycle of *B. bigemina* in the tick. They constitute the multiplicative phase of the life-cycle by means of which enormous numbers of the infective sporozoites are produced. Their migratory activities carry them into practically all parts of the body of the tick embryo so that it is certain that some of the salivary acini become infected. Since the sporokinetes are intra-cellular parasites, repeated cell division in the embryonic tissues undoubtedly aids in distributing them throughout the embryo. If it is borne in mind that the anterior ectodermal cell mass and the mesoderm are the most actively proliferating tissues of the tick embryo, it becomes clear why *Babesia* becomes concentrated in the salivary glands and the connective-tissue sheaths of the muscle bundles of the tick larva.

The sporozoites are the smallest as well as the most numerous forms of *B. bigemina* in the tick. In infected tick larvae some of the salivary acini become almost solid masses of *Babesia*. The sporozoites are most numerous in the loose mesenchyme cells which sheath the organs of the viscera; when they are especially numerous it is difficult to see the outlines of the parasites, but the more intensely staining nuclei of the sporozoites give the host cells the appearance of being filled with small chromophile granules. The sporozoites of *B. canis* were described by Christophers (1907), and Koch (1906) figures the sporozoites of *Babesia bigemina*.

RELATIONSHIPS

Babesia has long interested the protozoologist as well as the economic parasitologist. Whereas the latter has been primarily interested in the therapy and control of the diseases caused by the piroplasms, the former has sought to throw some light upon the relations of the Haemocytozoa to the flagellates.

Schaudinn (1904) pointed out the constancy of the apical chromophile granule in the trophozoites of *Babesia bigemina*, and suggested that this structure is homologous with the blepharoplast of the Flagellata. Breinl and Hindle (1908) described flagellated forms of *B. canis* from the blood of dogs, but subsequent investigation has failed to show the possession of flagella by *Babesia* at any stage in its life history. Dennis (1930) pointed out that *B. bigemina* is flagellate-like in its method of binary fission as well as in the possession of a blepharoplast and rhizoplast, but in the absence of a flagellated stage or the demonstration of sexual phenomena in the invertebrate host, more definite assignment has been contingent upon further investigation of the life cycle in the tick.

In view of the life-cycle of *Babesia bigemina* that has been described above, the organism must still be considered a sporozoan. The entire development of *Babesia bigemina,* however, follows a simple procedure which would place it among the less specialized, i.e., phylogenetically younger of the Sporozoa. Even the type of sexual reproduction exhibited is of a very primitive nature, for the production of isogametes occurs in *Chlamydomonas,* one of the more primitive Phytomonadina, though sexual phenomena are unproved in other Mastigophora. The presence in *Babesia* of features of both the Mastigophora and Sporozoa makes speculation interesting. At least the morphology and life-cycle of *Babesia* give a clue to the origin of the Haemocytozoa, and provide a beautiful hypothetical ''transitional'' form between the haemoflagellates on one hand and the more specialized Haemosporidia on the other.

SUMMARY

The life-history and anatomy of *Margaropus annulatus* are reviewed, and an outline of the embryology is presented. From this description it is apparent that *M. annulatus* is ideally designed for its function as the biological vector of *Babesia bigemina.* Its suitability may be summarized as follows:

(1) The protozoan parasites are limited to the circulatory system of the bovine host, and the tick feeds on nothing but the blood of the host.

(2) The period of feeding of *M. annulatus* is long and since all of it is spent on a single animal the opportunity for taking in the *Babesia* is greatly enhanced, even though the host is but a ''carrier'' and the parasites are scarce in the peripheral blood.

(3) The gut of the tick is blind so that any parasites ingested and not lysed by the digestive ferments are retained in the gut and tend to accumulate, thus increasing the opportunity for gametes to become associated.

(4) The fact that *M. annulatus* normally attaches to but one host during its lifetime makes it obligatory that the parasites be passed on through the agency of the offspring.

(5) The extremely intimate relationship of the digestive tract and the reproductive organs makes it almost a foregone conclusion that any parasites leaving the gut and entering other tissues will invade the latter and come to occupy the ova.

(6) As has been shown, certain stages of *Babesia* migrate throughout the tissues of the developing tick, and since much of the embryonic

cell mass contributes to the formation of the salivary glands, it is almost inevitable that some of the parasites should come to lie in these structures, whence they may be transferred to a new host during the feeding processes of the seed tick.

The life-cycle of *Babesia bigemina* has two distinct phases: (*a*) an asexual cycle in the vertebrate host where multiplication takes place in the red blood corpuscles by binary fission; and (*b*) a simple sexual cycle in the tick.

When blood which is infected with *B. bigemina* is taken into the gut of the tick, many of the intracorpuscular parasites are soon freed. Certain of these normal-appearing parasites become transformed into gametes through growth and slight structural modification. The gametes are motile vermicule-like bodies which show no differentiation between the sexes. The gametes become associated in pairs, the individuals of which eventually fuse to form the zygote. The zygote becomes a motile oökinete which passes through the thin wall of the gut and penetrates the contiguous reproductive organs. The ova of the tick are invaded by the oökinetes which round up and grow to form sporonts. The sporont secretes a cyst within which it divides to form naked sporoblasts. The sporoblasts form multinucleate sporokinetes which migrate, and are carried by cell proliferation, throughout the tissues of the developing tick; some of the sporokinetes come to occupy the anlagen of the salivary glands. The sporokinete undergoes fragmentation to form the minute infectious sporozoites.

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EXPLANATION OF PLATES

All figures drawn with the aid of a camera lucida.

PLATE 23

Fig 1. Median sagittal section through a mature ovum of *Margaropus annulatus*, showing the disposition of yolk and the germinal vesicle. Iron-haematoxylin stain × 130.

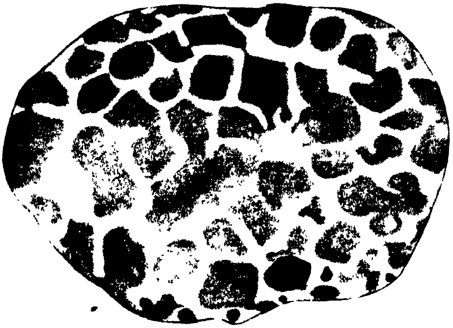
Fig. 2. Median sagittal section showing blastoderm and the proliferation of the endodermal ridge; anterior region is to the left. Iron-haematoxylin stain × 56.

Fig. 3. Sagittal section showing the proliferation of the anterior ectodermal cell mass. Iron-haematoxylin × 56.

Fig. 4. Transverse section through the fourth pair of limb-buds, showing the distribution of the mesoderm, and the endodermal ridge. Iron-haematoxylin × 56.

Fig. 5. Left half of transverse section through the anterior region of a newly-hatched tick larva, showing the relation of the salivary gland anlagen to the brain mass; also the sparse distribution of the endoderm just dorsal to these structures. Iron-haematoxylin stain × 56.

Fig. 6. Median sagittal section through an embryo just previous to hatching. Shows the relationship of the digestive tract to the brain, the appearance of the excretory vesicle, and the dorso-ventral muscles; the gut has not formed. Delafield's stain × 130.



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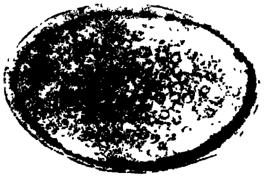

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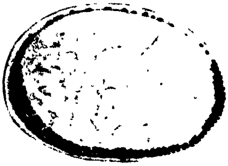

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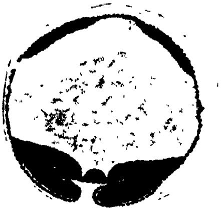

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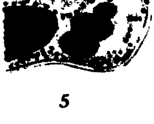

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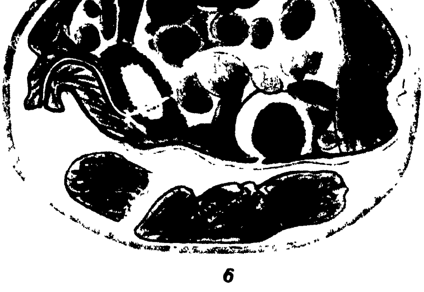

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PLATE 24

Figs. 7, 8, and 9. Trophozoites of *Babesia bigemina* in red blood cells of the bovine host. Giemsa's stain × 4360.

Figs. 10, 11, and 12. *B. bigemina* stained with iron-haematoxylin × 4360.

Fig. 10. Small sporozoite i.e., young trophozoite, in a red blood cell.

Fig. 11. Two small *B. bigemina*, probably resulting from the division of such a form as the one in fig. 10.

Fig. 12. Two large trophozoites of *B. bigemina* showing structure of the nucleus, and the blepharoplast and rhizoplast.

Figs. 13–18. *Babesia bigemina*, trophozoites, as they appear in the gut of the tick. Iron-haematoxylin × 2562.

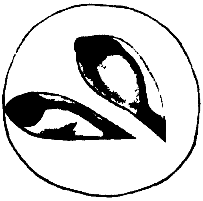
Figs. 19–20. Transformation of trophozoites into the isogametes. Iron-haematoxylin × 2562.

Fig. 21. Typical vermicule-like isogamete. Iron-haematoxylin × 2562.

Figs. 22–24. Herpetomonad-like nonflagellated forms seen in the gut of the tick. Iron-haematoxylin × 2562.

Figs. 25–26. Larger forms seen in the gut, showing an apical chromatin granule and traces of a fibril. Iron-haematoxylin × 2562.

Figs. 27–29. Club-shaped bodies of *B. canis*. Interpreted by Christophers as dividing forms. Redrawn from Christophers (1907) for comparison. Giemsa's stain × 1000 (?).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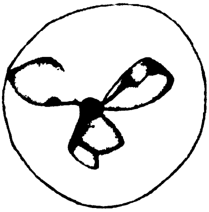
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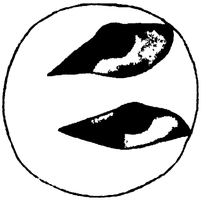
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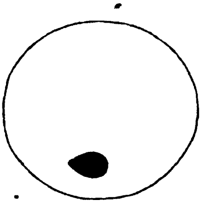
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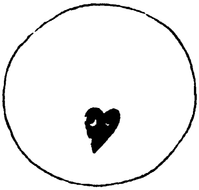
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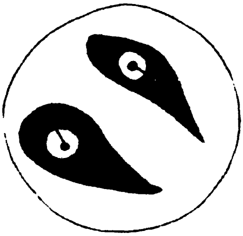
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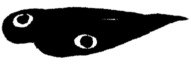
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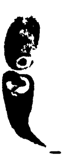
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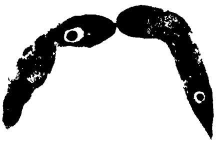
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PLATE 25

All figures except fig. 32 stained with iron-haematoxylin × 2562.

Fig. 30. Two specimens of *Babesia bigemina*, typical of the forms most common in the gut of the tick.

Fig. 31. Associated isogametes. Beginning of syngamy.

Fig. 32. Associated gametes observed in a fresh smear of the contents of the gut of the tick.

Figs. 33–34. Later stage of syngamy, seen in stained smear of contents of the gut.

Figs. 35–36. Oökinetes of *B. bigemina* in the gut of the tick.

Fig. 37. One of a large number of oökinetes in a section of a mature tick ovum.

Fig. 38. Large oökinete showing an anterior cup. From a smear of tick ova.

Fig. 39. Parasites of unknown relationship observed in smear of tick ova.

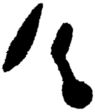
Fig. 40. Part of an oökinete in a section of an ovum of the tick.

Fig. 41. Two oökinetes in a large embryonic cell of a nymph.

Fig. 42. Oökinete in section of ovum of tick.

Fig. 43. Sporont imbedded in the yolk of ovum of tick.

Fig. 44. Increase in number of nuclei in a small sporocyst in the yolk of ovum of tick.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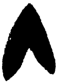
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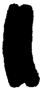
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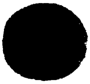
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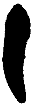
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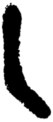
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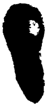
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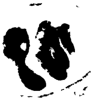
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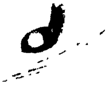
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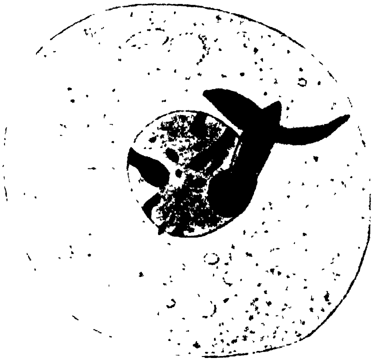
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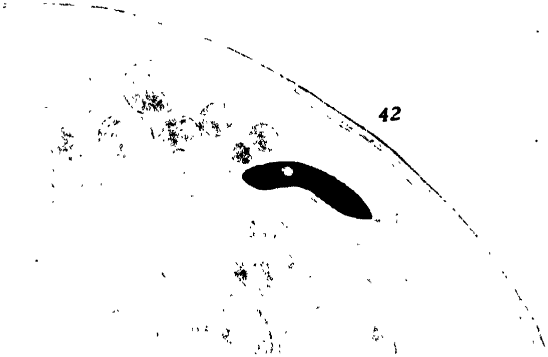
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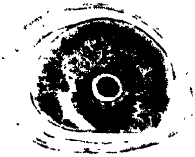
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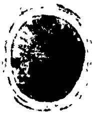
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PLATE 26

Fig. 45. Sporoblasts of *B. bigemina* from smear of early embryonic stage of the tick. Iron-haematoxylin × 4360.

Fig. 46. Sporoblasts in section of tick ovum. Iron-haematoxylin × 4360.

Fig. 47. Sporokinetes in a smear of heavily parasitized tissue of tick larva, showing characteristic shapes. Iron-haematoxylin × 4360.

Fig. 48. Large and rather atypical *B. bigemina* as seen in a single focal plane of a heavily infected salivary acinus of a nymph. Delafield's haematoxylin × 2562.

Fig. 49. Typical sporozoites of *B. bigemina* as seen in a single focal plane of a heavily infected portion of a salivary anlage of the tick. Delafield's haematoxylin × 4360.

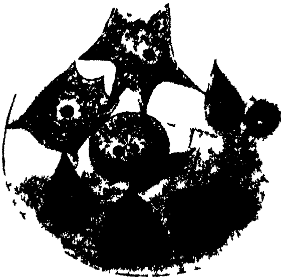

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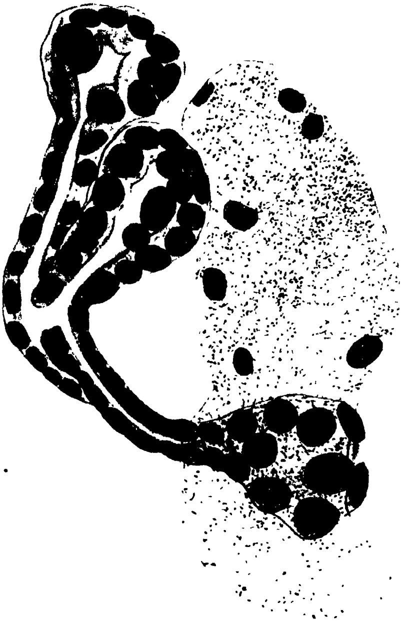
PLATE 27

Fig. 50. Heavily parasitized salivary gland tissue as seen in a squash preparation of a seed tick, stained with Giemsa. The stippled appearance of the lobe to the right is due to the nuclei of the sporozoites which are so numerous that the outlines of the individuals are seldom distinguishable × 500.

Fig. 51. Sporozoites of *B. bigemina* in the sheath of a muscle fiber of a tick larva. Giemsa × 1281.

Fig. 52. The right end of a transverse section of a heavily parasitized seed tick, showing the distribution of *Babesia* in the cells of the anlage of a gut diverticulum, in the blastoderm, and in the sheath of the dorso-ventral muscles. Salivary tissue heavily stained; cuticle of the tick is not shown. Delafield's haematoxylin × 1083.

Fig. 53. A lobe of embryonic salivary gland, heavily parasitized with sporokinetes of *B. bigemina*. Squash preparation; iron-haematoxylin × 800.



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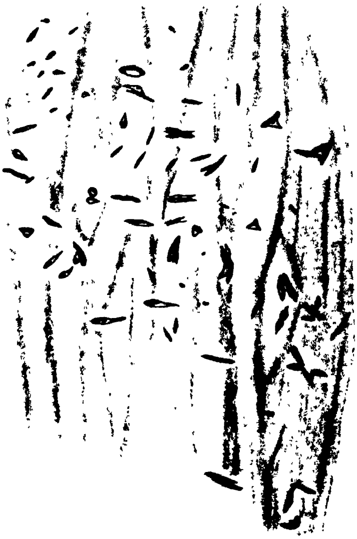

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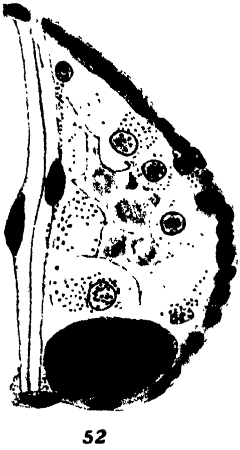

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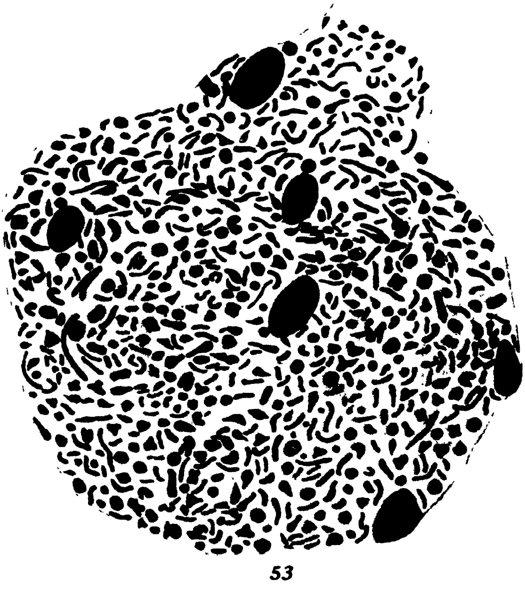

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PLATE 28

A schematic diagram of the life-cycle of *B. bigemina*.

Figs. 1–6. The cycle of *B. bigemina* in the blood of the bovine host, showing the method of binary fission.

Fig. 7. *B. bigemina* which have just been taken into the gut of the tick.

Fig. 8. Trophozoites of *B. bigemina* which have been freed in the gut of the tick.

Fig. 9. The vermicule-like isogametes.

Fig. 10. Beginning of syngamy. Association of the gametes in pairs.

Fig. 11. Completion of syngamy.

Fig. 12. The motile zygote, or oökinete.

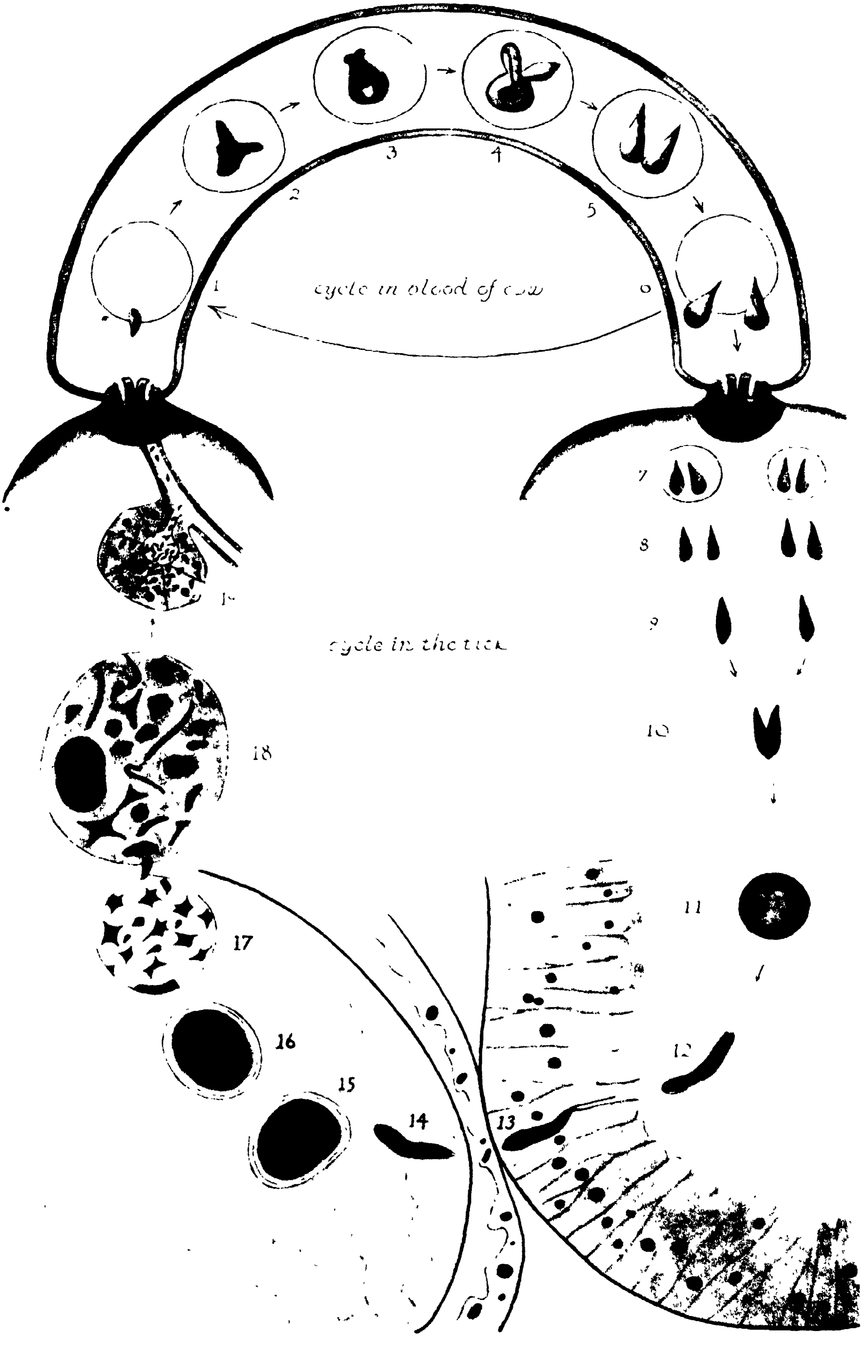
Figs. 13 and 14. The oökinete passes through the wall of the gut of the tick, through the oviduct, and enters the ovum.

Fig. 15. The sporont formed by the rounding-up and growth of the oökinete.

Figs. 16 and 17. Formation of sporoblasts.

Fig. 18. Sporokinetes in one of the large cells which are destined to form part of a salivary acinus.

Fig. 19. Sporozoites, formed by the fragmentation of sporokinetes, in the salivary gland (a single acinus shown) of the larva of the tick, whence they are transferred into the blood of a new host.


cycle in blood of cow
cycle in the tick

MORPHOGENESIS

BY

J. FRANK DANIEL

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INTRODUCTION

In the discussion of the genesis of organic form which follows, I wish to keep in mind the concept of an organism as a specific mould in space, as, for example, the space which would be occupied by the adult frog or the adult elephant. The adult form would be attained only after passing through a series of stages; in the frog these would be: zygote, blastula, gastrula, neurula, and larva. Each of these stages has its own form which is as peculiar to the frog as is the adult form itself. If we could cast a transparent mould of an adult and in this orient the zygote, the development of the individual could be followed through early differentiation and growth, and organogenesis, larval metamorphosis and later growth, until the mould with which we started would be completely filled.

During any stage in the life-history of the frog or of the salamander the mould would be filled with material which is *peculiar* to the species. This faithfulness of the specific material would obtain and would be demonstrable, for example, in pieces of the young gastrula of *Triton taeniatus* which had been transplanted to the nearly related *Triton cristatus*. These transplants in *Triton cristatus* may develop in accordance with the new position in the mould, but they would still remain true to their type. They would produce structures characteristic of *Triton taeniatus*.

The various stages might differ notably in complexity. It would be of great assistance to us if we knew at the outset to what extent the cytoplasmic materials of the egg are common stuff. In the frog, as an example, is the cytoplasm of the unfertilized egg isotropic or homogeneous as to its developmental capacity? Or does the visible pattern represent a chemical pattern as well? Do the pigment granules, localized as they are chiefly in the animal hemisphere, delimit an area that differs dynamically or chemically from the non-pigmented area? Similarly, do other physical inclusions, such as yolk granules, tend to alter their surroundings so that the cytoplasm containing these in great numbers, as for example, at the vegetative hemisphere of the egg, differs chemically from that of the animal area? If these areas differ in the unfertilized egg, then the cytoplasm could not even at this time be regarded as isotropic or as homogeneous throughout.

It will be remarked immediately that with the best technique at our command we are relatively ignorant of the early character of the cytoplasm. Since this is true, it is all the more important that attention be directed toward this point.

If we were filling an egg mould, then, with its own specific materials, the first thing we should need to know is: Are the materials of the egg with which we start common throughout? If not, is the egg composed of different materials which might take up specific positions in the pattern of the zygote or in more advanced stages of deveolpment? Such localizations might even be predestined for particular regions or organs in the embryo; they might, indeed, be organ-forming germinal areas, something like the "organbildende Keimbezirke" postulated by His (1874).

It would be insufficient, however, to know only the materials of the egg with which we start, for a marked shifting of the substances present may take place during early development. Does this shifting of material indicate the transportation of a particular kind of material for a particular part or structure in the pattern; or is the shifting a form-producing, morphogenic process? These two alternatives may be illustrated as follows: (1) That the first polar lobe in the mollusc, *Dentalium* is directly or indirectly responsible for the production of larval structures is shown by the fact that if it is removed no apical tuft or ganglion and no post-trochal area are formed (Wilson, 1904); (2) That the shifting of material may result in a visible pattern is indicated by puncture of an unfertilized egg of the frog, whereupon the gray crescent appears in which the future dorsal or axial organs arise (Brachet, 1911). In our discussion we shall consider numerous cases of migration of materials, a few of which appear to be a transportation of special materials for particular ends, while others indicate processes in the formation of pattern which may be evanescent, serving only in an early stage; in others these patterns may carry over to the larval or adult form.

A most important phase in morphogenesis is the production of new materials, new structures, new organs, and new systems. In early development, while there is an abundance of evidence of the rearrangement of old materials into new patterns, there is paucity of data on the formation of new materials. An experiment by Mangold (1923), however, gives us a close view of the changing of material into something new. By transplantation of a piece of presumptive ectoderm of the urodele *Triton* to a position in the dorsal lip of the blastopore, he demonstrated that the character of this material may be changed within a short time by its new surroundings so that it will now develop into notochord and (or) somites. In the hands of Spemann (1927) this experiment, as we shall see in a later paper, has been so stated as to give striking experimental evidence of epigenetic development.

Experimental studies, largely on the Amphibia, have shown that if the optic vesicle comes into contact with the inner layer of the epidermis, a new structure, the lens of the eye, will form. Just why this layer of relatively simple cells transforms into the more complicated lens is as yet unknown. That the formative stimulus is not specific within narrow limits is indicated by the experiment of Filatow (1925), who grafted skin from the toad *Bufo vulgaris* so that it came into contact with the optic vesicle of *Rana esculenta,* with the result that the optic vesicle of *R. esculenta* induced a lens from the transplant.

The periods during and immediately following gastrulation are the moments *par excellence* for the production of new organs and new systems. These we shall consider in later papers.

I. FERTILIZATION AS A FACTOR IN MORPHOGENESIS

Plates 29–32; text figures 18 and 19

If we regard fertilization as an interaction between male and female gametes, giving the zygote full capacity for development and inheritance (Lillie), we may consider it either as a process, that is, a series of processes; or we may study it in terms of its results. We are interested here primarily in the results of fertilization. The question is: What does fertilization contribute to specific organic form? In order to consider this question more effectively we shall confine our study to a few types around which a considerable body of information has accumulated, and keep before us the three criteria above mentioned: (1) the materials of which the egg is made; (2) the transportation and disposition of the materials during fertilization; and, finally, (3) the creation of new materials or the segregation of new or old materials into patterns which may or may not be of significance in or beyond the zygote.

A. MATERIALS OF THE EARLY ZYGOTE

The immature and unfertilized egg of the sea urchin (*Arbacia* or *Paracentrotus lividus*) is characterized by the presence of pigment granules of a reddish or brickish color in the superficial layer of cytoplasm. Within this pigmented layer is a sphere of grayish protoplasm; and in the upper part or animal half of this is the large clear germinal vesicle. After the germinal vesicle has broken down and maturation has been completed (fig. 1), the pigment still retains its position around the entire egg. By the time the spermatozoon has entered the egg the pigment has shifted to a horizontal band around the equator (*pg.*, fig. 3). Above this band an extensive area of cytoplasm now occupies the animal hemisphere, and below it a smaller clear area surrounds the vegetative pole (*Paracentrotus*). Superficially, then, the pattern of the young zygote is unlike that of the egg which has just matured in this stratification. In addition, the young zygote has the entrance cone of the sperm (*co.*, fig. 3) and a fertilization membrane (*fm.*) which is lifted away from the main body of the egg, especially in the area of the flattened animal hemisphere.

From a remarkable series of experimental studies Driesch concluded that the materials of the egg of the sea urchin at the beginning of fertilization, and even in early cleavage, are uniform throughout. Boveri (1901), however, observed that the three areas of the egg above indicated correspond to the primitive rudiments in the larva. The unpigmented animal half produces the ectoblast and its derivatives; the pigmented girdle forms the digestive tract; and the vegetative unpigmented cap forms the primary mesenchyme and larval skeleton. In a recent study Hörstadius (1928) has thrown serious doubt on the conclusions of Driesch by showing that in the egg of *Paracentrotus lividus,* animal and vegetative areas are unlike even at an early stage following sperm entrance in that the material in the animal hemisphere, if cut off, will develop as a resting blastula; that of the vegetative half, on the other hand, acts as an organizer and will produce a whole though small embryo.

The egg of the ascidian, *Styela partita,* before fertilization is composed of a peripheral layer of cytoplasm (*y.*, fig. 4) of a finely granular kind which carries yellowish spherules (mitochondria) uniformly distributed in it. Within this superficial layer is a second grayish mass of yolk-bearing protoplasm (*gy.*), The germinal vesicle (*gv.*), containing a clear substance, lies largely in the grayish yolk but has an excentric position toward the animal pole. If one could force the germinal vesicle to a central position these three differently colored areas would represent a concentric arrangement, the superficial layer with yellow spherules being outermost, the grayish yolk-bearing material median, and the clear germinal vesicle being central in position. In *Styela* there is evidence that the protoplasm is not common throughout, but contains certain organ-forming materials, some of which, as we shall see later, can be followed by their colors into particular areas and finally into particular organs or parts of the embryo.

In the egg of the frog, Von Baer (1834) showed that upper and lower hemispheres are readily distinguishable in at least two ways. Visibly, the upper hemisphere (*an.*, fig. 7) is deeply pigmented, and the lower area (*vg.*) is whitish or yellowish in color. Moreover, in the floating egg the pigmented area remains up and the whitish area down, indicating that the two differ also in specific gravity. Remak (1855) further concluded for the fertilized egg that the upper or "animal" hemisphere transforms into the more active, outer layer from which the skin, the nervous system, and the special senses of the adult are derived, and that the lower or "vegetative" hemisphere gives rise to the more inert, inner layer from which the primitive digestive tract and its derivations arise.

In a section through the unfertilized egg of the toad Bambeke early (1876) showed that the layer of black pigment surrounding the egg is superficial in position, and that under this, in the animal area, is a cap of light brown color. When this cap is viewed in section through animal and vegetative poles it appears as a crescent, the horns of which terminate near the equator. From the thickest part of the crescent near the animal pole a blob or club-shaped extension of this light brown material hangs well down toward the center of the egg, so that the whitish yolk at the vegetative hemisphere takes the form of a thick walled bowl filled with this blob. Yolk granules present throughout the cytoplasm of the egg are particularly compact in the lower white area.

B. Movement of Materials in the Zygote

Following the breaking down of the germinal vesicle and the giving off of the polar bodies in the egg of the sea urchin, *Paracentrotus,* and accompanying or perhaps even preceding fertilization, there is a marked shifting of the superficial reddish pigment to a new position just below the equator. Here it collects as a horizontal band or girdle (*pg.,* fig. 3) around the whole egg, leaving an extensive clear area above and a small clear area below in the regions of the vegetative pole (*Paracentrotus*).

The movement of material within the egg of *Styela partita* at the time of maturation and fertilization has been studied in detail by Conklin (1905). In the gamete, the germinal vesicle (*gv.,* fig. 4) breaks down, and in case the egg is not fertilized the clear stuff of the germinal vesicle, as well as the gray yolk-bearing protoplasm, and the yellow materials retain their position in the egg until disintegration takes place. If, on the other hand, the sperm enters the egg and fertilization ensues, a remarkable movement and relocalization of materials take place. The layer of protoplasm containing the peripheral yellow spherules streams toward the lower pole (fig. 5), where it takes a horizontal position somewhat like that taken by the reddish granules in the egg of the sea urchin, *Arbacea.* According to Conklin, there is formed just above this horizontal band of yellow also a horizontal band of the clear material from the germinal vesicle. A second shifting of this material for the embryonic pattern then occurs, by which the yellow and the clear bands migrate with the sperm pronucleus and aster to a position which will be posterior in the future embryo (*yc.*, fig. 6). Here the yellow material forms a crescent just below the equator, and the clear substance is chiefly withdrawn from the surface and comes to surround the sperm pronucleus and aster. A bit of it remains superficially in the center of the yellow crescent. Most of the clear material now moves with the sperm nucleus to the center of the egg.

For the frog's egg Newport (1854) was the first to ascertain that the sperm cell penetrates the egg by its own (?) movements, and Bambeke (1876) saw the triangular trail of pigment in the egg of the toad, which he surmised was the sperm path. Roux analyzed the sperm path of the frog (*sp.*, fig. 17) into two components. The first of these, the penetration path, extends from the meridian of penetration along a radius toward the center of the egg. The second component, in which the sperm pronucleus meets the egg pronucleus, is the copulation path. In case the egg pronucleus lies opposite the entrance point of the sperm these two components lie in the same plane, but when the egg pronucleus lies outside of this plane the copulation path makes an angle with the penetration path as sperm pronucleus moves toward egg pronucleus. As the two pronuclei approach there is evidence of marked cytoplasmic disturbances by which the central core or blob of brown material is displaced toward the side of the egg opposite sperm penetration (see fig. 17).

Two hours after the entrance of the sperm into the egg of *Rana fusca* Roux observed a notable shifting of pigment from the side of the egg opposite the place where the sperm had entered. As a result of this disappearance of pigment an area appears which is lighter in color and crescentic in shape (*gc.*, fig. 9). If this crescent be bisected, dividing it into right and left halves, the plane of bisection will be approximately along the meridian 180 degrees from the point of sperm entrance. The crescent itself is the important gray crescent which localizes the dorsal or axial organs of the embryo.

In a more detailed study of a sagittal section through the egg it is observed that the pigment on the side where the sperm enters forms a thick and dense layer (fig. 17*a*), while on the sides of the gray crescent it remains as a thinner and less compact layer (fig. 17*b*). It was Roux's opinion that the pigment in the latter case had migrated inward from the surface. Weigmann (1927), in a recent restudy of the shifting of pigment in *Rana fusca,* finds that a net-like loosening of pigment takes place in the area of the future gray crescent. Later, after the crescent is fully formed, clouds of pigment may be found deeper in the cytoplasm. In surface view Weigmann found that the gray crescent (*gc.*) may appear as in figure 8, where the pigment is cleared in alternate patches. In other cases he observed on the surface of the egg brown parallel stripes as if the material were streaming across the vegetative area.

A series of interesting studies on the shifting of pigmentation in the egg of *Discoglossus pictus* has recently been made by Wintrebert (1931). If seen from the animal pole the clear germinal area (*gr.*, fig. 11) occupies a central or polar position an hour after the sperm has entered the egg. During the next two hours, while the sperm nucleus is approaching and beginning fusion with the egg pronucleus, this area migrates ventrally until it comes to occupy a position (*gr.*, figs. 12–13) on the ventral margin of the superior polar plateau. At an hour and a half to two hours there extends posteriorly from the germinal area over what will be the dorsal quarter of the zygote pigmented fan-like rays, terminating in the gray crescent (*gc.*, fig. 12). Within another hour rays also extend laterally from the raphe made by the migration of the germinal area forward (ventrally). At this time, in addition, there is a heavy mass of pigment collected just in front of (ventral to) the germinal area which streams ventrally toward the equator. Below the equator a black shield forms (*bs.*, fig. 13) due to the ventral migration of pigment as the egg rolls forward. Between the germinal area and the lower margin of the black shield concentric rays (*pg.*), parallel to the sagittal plane, stream to the vegetative area and upward into the white shield (*ws.*).

In the urodele amphibians, Axolotl and *Pleurodeles,* there is at the time of the appearance of the gray crescent a general shifting of materials, so that the egg rolls toward the future ventral side of the embryo, as it does in *Discoglossus,* lifting the crescent to a position above the equator. By placing a vital dye, for example, at or near the equator of the egg of Axolotl with the animal pole upward, the animal pole is seen to shift with relation to the equator. In such a case, if the dye be placed on the equator and, by chance, within the area of the crescent, the crescent would be seen to rise above the horizon. Banki (1928) shows that in the egg of Axolotl the crescent appears in the region of the equator, four to six hours after fertilization. He has come to the conclusion that grayish stuffs just under the superficial pigment and above the equator stream toward the vegetative pole. Through the displacement of material the gray crescent is produced and appears above the equator as the whole egg rolls forward (ventrally) to gain a new equilibrium.

The movement of materials in the zygote of *Triturus torosus* (figs. 14–16) is similar in several respects to that of *Discoglossus*. After sperm entrance the upper part of the animal hemisphere flattens out (figs. 14–15) and appears to be more or less in a sol state. The germinal area (*gr.*) (Richtungsfleck) moves toward the future anterior part of the organism and the pigment now in front of it sinks ventrally below the horizon of the egg as a pigmented shield (*bs.*, fig. 16). Opposite this a light area (*gc.*) rises above the horizon on the future dorsal side of the embryo in the form of a crescent. This dorsal region early appears to be very mobile and readily flows toward the germinal area.

C. THE PATTERN OR MOULD OF THE COMPLETED ZYGOTE

The horizontal band of reddish pigment in the egg of the sea urchin (fig. 3) which forms at the end of maturation or beginning of fertilization serves as a landmark separating the beginning zygotic pattern into three zones: an upper clear area in the animal hemisphere in which the female pronucleus (*n.*), lies; a lower and smaller clear region surrounding the vegetative pole (*Paracentrotus*); and the pigmented girdle (*pg.*) around the egg separating the two clearer areas. The pigmented area occupies the upper part of the vegetative hemisphere and may extend a short distance above the equator into the animal half.

That the pigment itself is not essential to development is indicated in two ways. First, it has been shown (Boveri, Hörstadius) that it varies greatly in amount in different eggs of *Paracentrotus*. In some it is profuse; in others it is evident but not abundant; while in still others it is entirely lacking. Despite the fact that the pigment may be entirely absent, the egg undergoes normal development. Secondly, by centrifuging at a rate of 10,000 revolutions per minute, Lyon (1907) has shown that the materials of the egg of *Arbacea* (fig. 2) can be separated within a short time into four zones. From the centripetal pole out these are: (1) light oils or fats (fig. 2), (2) a clear zone in which is the egg nucleus, (3) the yolk, and (4) around the outer or centrifugal pole the pigment. This stratification persists throughout cleavage and, although there has been a complete shifting of the pigment, normal development follows.

Externally the pattern of the late zygote of the sea urchin resembles that of the early zygote, but that the two differ is shown by the studies of Hörstadius on eggs of *Paracentrotus*. Eggs were fertilized and then separated meridionally, some within the first quarter of an hour after fertilization, others within the second quarter, and so on by quarters until the end of the hour. A comparison of those separated early after sperm entrance with those separated later shows that while 68.9 per cent of those separated within the first quarter develop as wholes, only a small percentage, 9.8 per cent, separated an hour after sperm entrance produce wholes. During the first and second quarters after sperm entrance none will develop as halves, but by the end of the fourth quarter, that is, by the end of the hour, 29.4 per cent develop as halves. This study indicates that a progressive differentiation has taken place during fertilization by which the completed zygote, although in appearance like the beginning zygote, is fundamentally changed. The change which has taken place can be judged, however, only by the later development. Halves fertilized and separated during the fourth quarter form only two micromeres in the sixteenth cell stage, showing that they are developing as halves.

The pattern of the complete zygote in *Styela* (fig. 6) seen from the left side consists of light gray material which, *following first cleavage,* represents an indistinct crescent like the gray crescent of the frog. It also takes the same position in relation to the sperm nucleus, but not in relation to entrance point of the sperm, and gives the same derivatives, dorsal organs of the embryo. Opposite this light crescent is the pronounced orange yellow crescent (*yc.*), above which is a crescent of clear material, the two marking the posterior region of the zygote. Above and between the anterior light gray *presumptive* crescent and the posterior yellow and clear crescents is a transparent hyaloplasm. Below and between the two is the grayish yolk-bearing material (*gy.*). "These different areas of cytoplasm are specific factors in the development of particular tissues and organs, and in this sense they are organ-forming substances" (Conklin, 1931, p. 77).

The spherules of the yellow crescent normally accompany the crescent through the migrations which we have described. Conklin

(1905) has shown that if the zygote be centrifuged so as to throw the yellow crescent into abnormal positions, the displacement of this material results in an embryo with abnormal parts. Recently (1931) Conklin has shown, however, that the yellow granules may be displaced without displacing the granular plasma which normally gives rise to the musculature.

In the Amphibia several distinguishing features characterize the completed zygote. In the anuran *Discoglossus* the germinal area (*gr.*, fig. 13) at this time occupies a position at the anterior (ventral) margin of the superior plateau instead of at the animal pole, as in the beginning zygote (fig. 11). Moreover, through the forward (ventral) rotation of the egg, two other features appear: A pigmented shield (*bs.*) ventral to the germinal area now extends below the horizon, and on the opposite (dorsal) side of the egg the pigment has lifted up so as to form a white area (*ws.*) comparable in extent to the black submerged area and contiguous with the gray crescent (*gc*).

In the urodele, *Triturus torosus* similar general features characterize the completed zygote. At this stage the egg is still flattened, with the chorion (*ch.*, fig. 16) high above its surface. The germinal area (*gr.*) has moved forward and the pigmented shield (*bs.*) is a marked feature. The gray crescent (*gc.*) is usually well defined and may possibly include more than it does in a type like *Rana fusca.*

In a small percentage of the eggs observed (3 per cent to 4 per cent) pigmented stripes or bands may take up positions producing patterns in the late zygote which, although not so regular as those shown in the diagram for *Discoglossus,* are in general like them. The most frequent pattern occurs in the area of the pigmented shield. In case first cleavage is sagittal, this pattern may be bilateral. Two other patterns of bands have been observed. One of these was composed of stripes at right angles to the course which the germinal area had traversed. The other consisted of sixteen to eighteen concentric stripes running parallel with the sagittal plane. In addition to these patterns, I have observed only occasionally an indication of pattern in the region between the crescent and the germinal area.

The most outstanding single feature of the amphibian zygote is doubtless the gray crescent (*gc.*) characteristic of the Anura and of certain urodeles, Axolotl, *Pleurodeles, Triturus torosus* and, in certain cases, *Triton.*

The crescent (figs. 9, 10, 13, and 16) varies somewhat in its horizontal extent and vertical position. The arms or horns in some eggs may extend entirely around the equator, although they usually fall short of this. The vertical position varies slightly as regards the horizon. In *Rana* it is relatively near the equator. In *Discoglossus* it is high. The greatest vertical width of the crescent represents the sagittal plane or the midline through the back of the embryo and separates the crescent into right and left halves corresponding to the bilateral symmetry of the embryo. The head end of the embryo points toward the animal pole, but only in the urodeles does it actually reach that position.

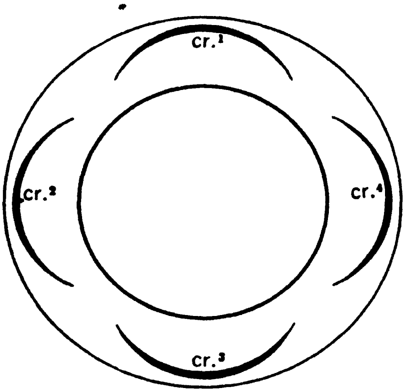
A section through the late zygote of the frog by Schultze (fig. 17) shows the crescent in relation to the general internal topography of the egg. The superficial cap of pigment extends over the upper hemisphere and well past the equator on the lower hemisphere. There is a small upper and a large lower (*vg.*) unpigmented area. Surrounding the white area in the upper hemisphere the protoplasm is of a light brown color (*br.*) like that shown by Bambeke in the toad's egg. This brownish material likewise takes about the same form as in the toad. In section it is seen to extend down the sides of the egg and to have a central club-shaped mass or blob extending into the white yolk. A marked internal feature of the late zygote of both is the displacement of the brown cytoplasm, giving it the appearance, as is shown in figure 17, of having shifted its position in advance of the sperm path.

It would appear from the above observations that the gray crescent is localized in relation to the point of sperm entrance. But how far is fertilization able to fix the crescent, and consequently to orient the embryo?

In addition to observing that the gray crescent in *Rana fusca* forms opposite the pigmented penetration path of the sperm, Roux, following Newport (1854), placed sperm cells either with a fine brush or by means of a silk thread on different meridia of the egg. In this way he was able to demonstrate experimentally that the crescent forms opposite the point of sperm penetration.

Herlant (1911) found that if two spermatozoa instead of one enter the egg, the crescent does not form opposite the entrance point of either, but midway between the meridia opposite the points of entrance of the two (fig. 19). For example, if we assume that the point of penetration of one sperm be on the first meridian and its meridian opposite is 180°, and the point of entrance of the second sperm be on meridian sixty with 240° the meridian opposite, the crescent in this case would appear with its widest vertical part near meridian 210°; that is, half way between 180° and 240°.

From the results of Roux and Herlant we may think of the crescent in the fertilized egg of *Rana fusca* as potential at any position in an equatorial band of material encircling the egg. If we view the egg from the vegetative area (fig. 18), this band of potential gray crescent would appear near the equator in the position later occupied by the germ ring; and the definitive crescent might arise in this band as in cr^1-cr^4 or with its greatest vertical width on any other meridian. Since any part of this band could act as a center around which the crescent could arise, it may well be that that part of it is so determined which lies opposite the disturbance produced by the point of sperm entrance and the deep penetration path.



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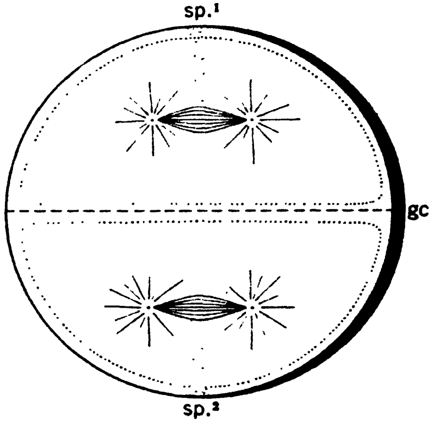

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Fig. 18. Diagram giving some of the possible positions (cr^1-cr^4) of the gray crescent in the frog's egg.

Fig. 19. Diagram showing entrance of two spermatozoa (sp^1-sp^2) in relation to the gray crescent (*gc.*) in the egg of *Rana fusca* (Herlant).

Activation of the same egg by puncture gives a different result. Bataillon (1910) has shown that some of the eggs punctured with a fine stylet will develop a gray crescent apparently similar in form in all respects to the crescent of fertilized eggs. Brachet (1911) found that about 10 per cent of the eggs punctured are activated, and that the crescent formed by this activation differs from that produced by normal fertilization in one essential point: it is not fixed or localized opposite the point of puncture. Indeed, following puncture the greatest vertical width may occur on any meridian. It may even be on a meridian near the point of puncture (*gc.*, fig. 10). From this it appears that while superficial puncture is capable of calling forth the gray crescent, it does not localize it.

Banki (1927) has shown for the egg of the urodele Axolotl that the spermatozoan may enter any point on the surface of the egg. If entrance be made through the dark area, it leaves a pigmented pit or funnel which serves as a landmark of penetration even up to the end of blastulation. The crescent which appears may frequently be opposite the point of sperm entrance. But this is by no means universal. Indeed, the widest part of the crescent may occur on any meridian of the egg and in any possible position as regards sperm entrance. In Axolotl fertilization is but little more effective in localizing the crescent than is puncture in *Rana fusca.*

The Anura and Urodela, then, which we have considered, present a series as to the localization of the crescent with reference to the entrance point of the spermatozoan. In the anurans thus far studied the crescent is localized opposite the point of entrance of the spermatozoan. In the same types, while puncture may call forth a gray crescent, it fails to localize it in relation to the point of puncture. In the urodeles, Axolotl, *Pleurodeles* and *Triturus torosus,* the crescent may form opposite the meridian of penetration, but it is not limited to that position.

In a recent study of the egg of *Triturus torosus* I have been impressed with the ease with which the pattern of the zygote can be disturbed by tilting the egg so as to cause the cytoplasm of the animal hemisphere to flow in a different direction. How far mechanical disturbance of this sort is capable of producing permanent alteration or reversal in the pattern is a problem open to the experimental method. By studies of this type we should be able to determine whether the crescent, as a part of the pattern, can be obliterated and a new one formed. If a new one could be produced then we might be able to ascertain something of the causes underlying its induction.

To what extent can the crescent of the zygote be considered as a center of organization?

Moszkowski (1903) first showed that if the early crescent be injured by means of a hot needle, the dorsal organs of the embryo, medullary plate, notochord, and myotomes, develop abnormally or are absent, and Brachet (1911) later demonstrated that puncture of the anterolateral, convex surface of the crescent produces abnormality in, or loss to, one-half of the medullary plate; while injury to the posterior and concave part causes injury or loss in the notochord. These experiments indicate that the materials for the medullary plate and the notochord are early localized in the crescent. The degree of their determination is uncertain. The median part of the crescent in the blastula, moreover, Brachet regards as a center of organization which acts first by directing the movements of the rudiments and then by calling forth their morphological differentiation.

In the urodeles, in which the dorsal lip of the blastopore is the organizer of Spemann, the dorsal organs are not concentrated within the crescent and are labile to a much later stage of development.

SUMMARY

The organism is considered as a specific mould in space, constructed stage by stage during development. The stage here especially considered terminates with complete activation, and the changes during this series of events are studied with reference to the contribution which they make to the specific organic form of this stage.

In the echinoderm, *Paracentrotus lividus,* the shifting of the visible pigmented pattern takes place early, so that accompanying or preceding sperm penetration the egg has the same visible features, that is, a horizontal pigmented band separating upper and lower clear areas, that it presents in the completed zygote. Hörstadius, however, shows that the egg has undergone a profound though invisible change, as can be judged by its future development.

In the ascidian, *Styela partita,* a marked shifting of visible pattern follows the entrance of the spermatozoon. Whether this movement with a relocalization of stuffs produces new materials is not known, but it results in four areas in the completed zygote, which, as specific factors in the development of particular tissues and organs, may be regarded as organ-forming.

In the Amphibia a number of features appear during the zygote stage. The most marked of these is the gray crescent. In the Anura the crescent arises in association with movement of materials and the clearing of this important area opposite sperm penetration. In the Urodela there is little indication that sperm entrance localizes the crescent. In both the crescent gives bilaterality to the zygote and to the embryo. For the anurans Brachet holds that the dorsal organs, medullary plate, notochord, and somites, are concentrated in the crescent. In the urodeles at this early stage these rudiments are more dispersed and are not fully determined until a later stage of development.

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EXPLANATION OF PLATES

PLATE 29

Fig. 1. Mature egg of sea urchin, *Strongylocentrotus* (*Paracentrotus*) *lividus*, lateral view (Boveri). *f.*, funnel in the jelly; *n.*, female pronucleus; *pb.*, polar body. Superficial pigment stippled.

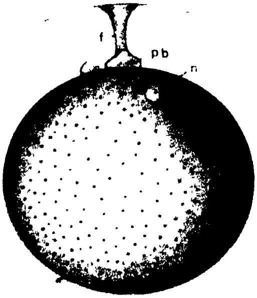
Fig. 2. Centrifuged egg of sea urchin, *Arbacia*, lateral view (Morgan and Spooner. *f.*, funnel; *l*, light oil droplets nearest centripetal pole; *2*, clear cytoplasm in which female pronucleus lies; *3*, granular or yolk layer; *4*, layer of pigment at outer or centrifugal pole.

Fig. 3. Fertilized egg of *Strongylocentrotus* (*Paracentrotus*) *lividus*, lateral view (Boveri). *co.*, entrance cone of spermatozoan; *f.*, funnel; *fm.*, fertilization membrane; *n.*, female pronucleus; *pg.*, horizontal band of pigment.

Fig. 4. Egg of *Styela partita*, showing the three nearly concentric layers (Conklin). *gv.*, germinal vesicle; *gy.*, gray yolk-bearing substance; *y.*, the clear outer layer carrying yellow spherules.

Fig. 5. Early zygote of *Styela partita*, showing the downward migration of yellow spherules upon entrance of sperm (Conklin).

Fig. 6. Late zygote of *Styela partita*, lateral (left) view (Conklin). *gc.*, prospective gray crescent which appears *after* first cleavage; *gy.*, gray yolk-bearing area; *yc.*, yellow posterior crescent.



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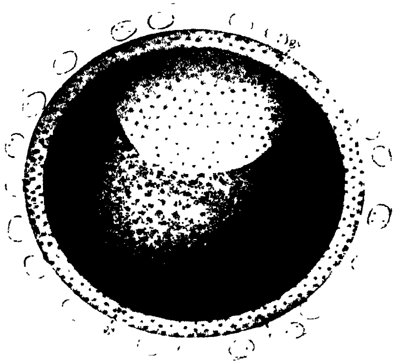

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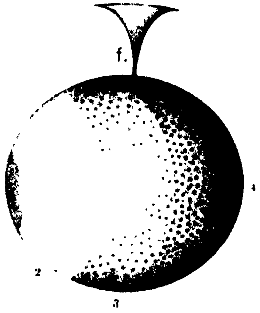

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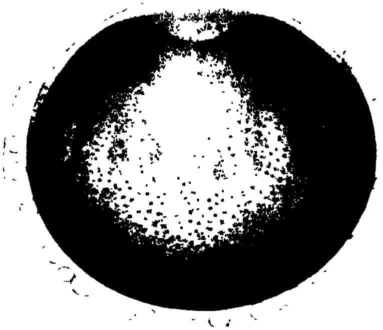

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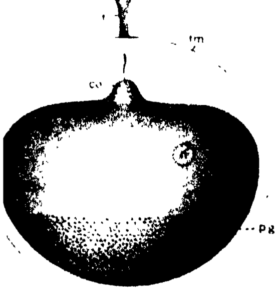

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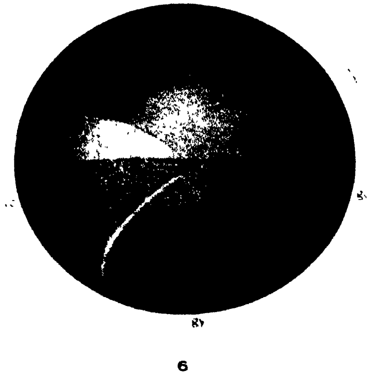

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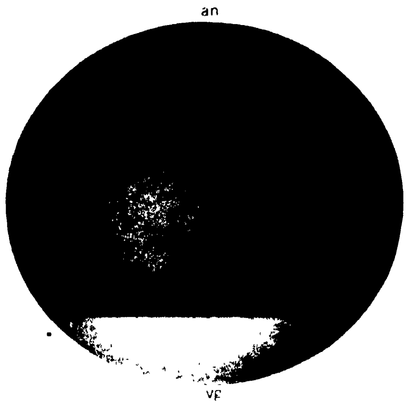
PLATE 30

Fig. 7. Egg of *Rana fusca,* lateral view (Jenkinson). *an.,* animal pole heavily pigmented; *vg.,* vegetative pole.

Fig. 8. Formation of gray crescent (*gc.,* light spots), *Rana fusca,* ventral view (Weigmann).

Fig. 9. Egg of *Rana fusca,* lateral view (Jenkinson). *an.,* animal pole; *gc.,* gray crescent; *vg.,* vegetative pole.

Fig. 10. Egg of *Rana fusca,* activated by puncture (Brachet). *gc.,* gray crescent; *po.,* exudate from puncture; *vg.,* vegetative area.



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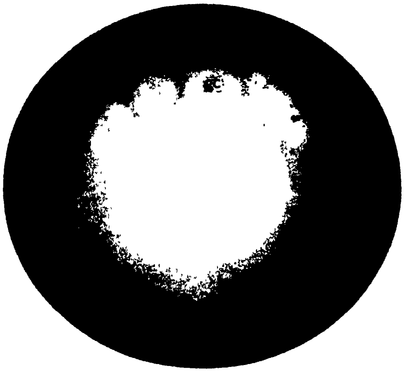

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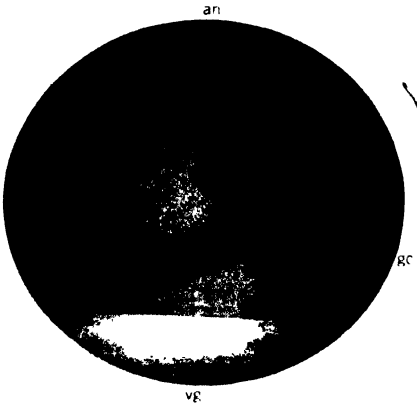

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PLATE 31

Fig. 11. Egg of *Discoglossus pictus*, seen from animal pole (Wintrebert). *gr.*, germinal area; *pb.*, polar body; *pl.*, superior plateau; *sp.*, sperm entrance.

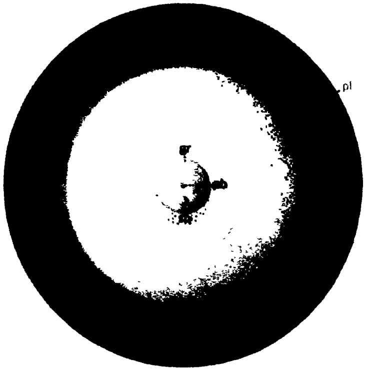
Fig. 12. Egg of *Discoglossus pictus*, seen from animal pole (Wintrebert). *bs.*, pigmented shield; *gc.*, gray crescent; *gr.*, germinal area now at anterior (ventral) border of superior plateau; *pg.*, fan-shaped, pigmented rays to gray crescent; *ws.*, white shield.

Fig. 13. Late zygote of *Discoglossus pictus*, lateral view, left side (Wintrebert). *bs.*, black shield below the secondary equator; *ch.*, chorion; e^{2}., original equator; *gc.*, gray crescent; *gr.*, germinal area; *pg.*, parallel pigment tracts from (*bs.*) toward (*gc.*); *ws.*, white shield of original vegetative area elevated above equator by forward (ventral) rotation of egg.

Fig. 14. Early stage of zygote, *Triturus torosus*, seen from animal pole, germinal area nearly central.

Fig. 15. Zygote of *Triturus torosus*, seen from animal pole. *gc.*, gray crescent; *gr.*, germinal area in which is second polar body.

Fig. 16. Late zygote of *Triturus torosus*, lateral view, left side. *bs.*, black shield; *ch.*, chorion ("fertilization membrane"); *gc.*, gray crescent; *gr.* germinal area.



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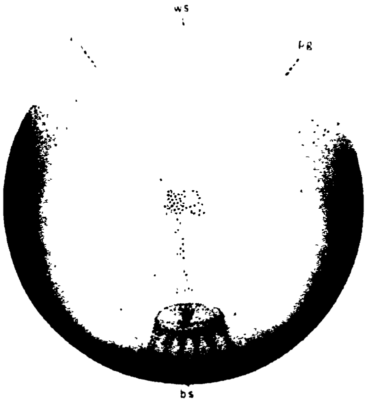

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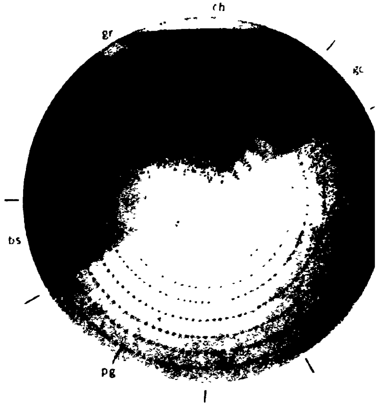

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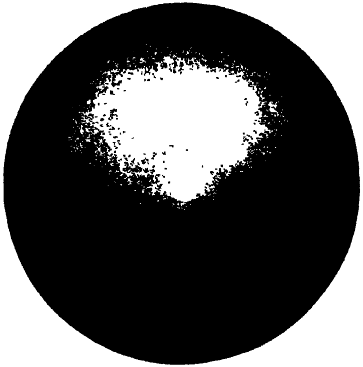

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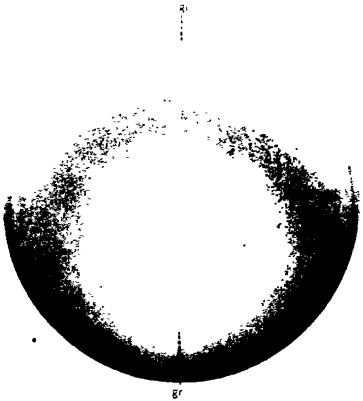

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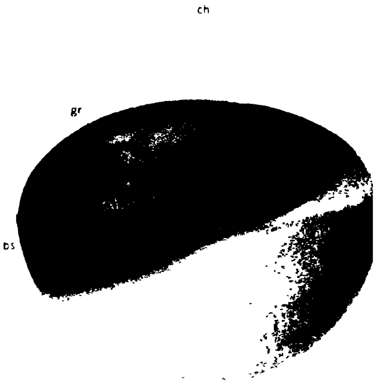

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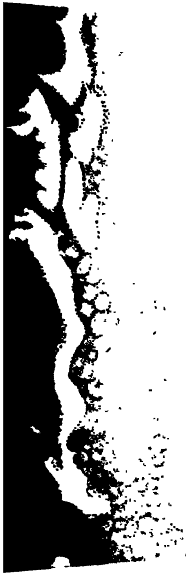
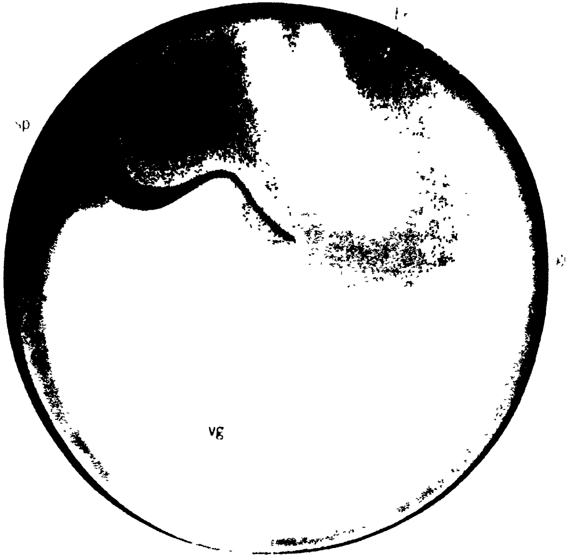
PLATE 32

Fig. 17. Section through egg of *Rana fusca* (Schultze). *br.*, brownish cytoplasm; *gc.*, gray crescent; *sp.*, sperm entrance; *vg.*, vegetative area.

Figs. 17*a* and 17*b*. Enlargements of section through surface of egg below sperm entrance (*sp.*, fig. 17) and through the gray crescent (*gc.*) respectively, showing relative density of pigmentation (Weigmann).



17*a*



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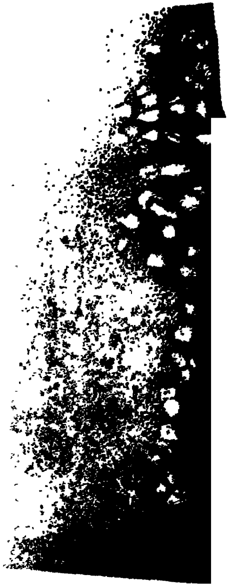

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MOVEMENT AND LOCALIZATION OF THE PRESUMPTIVE EPIDERMIS IN TRITURUS TOROSUS (RATHKE)

BY

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MOVEMENT AND LOCALIZATION OF THE PRESUMPTIVE EPIDERMIS IN TRITURUS TOROSUS (RATHKE)

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INTRODUCTION

The application of vital staining to the study of developmental mechanics in the Amphibia has yielded facts of such fundamental importance as to necessitate the revision of a number of concepts formerly considered basic. One of these facts concerns the movements and localization of materials during early development, which have been followed particularly by Goerttler (1925), Vogt (1929), Manchot (1929), Bijtel (1931), and Röhlich (1931). Any account of development in Amphibia is incomplete without their contributions. Most of the work done so far has been confined to a few forms, among which *Triton, Amblystoma, Pleurodeles,* and *Bombinator* are outstanding. I wish here to add similar work on *Triturus (Diemyctylus) torosus,* the common newt of the Pacific Coast of North America, which I undertook at the suggestion of Professor J. Frank Daniel.

MATERIALS AND METHOD

The experiments herein described were carried out by means of the vital staining technique described by Vogt (1925). From the total of 247 embryos stained at various stages of development a series of typical specimens has been selected for description.

A. THE RELATION OF CERTAIN BLASTOMERES TO PRESUMPTIVE EPIDERMIS

If stains are placed on the germinal area (*Richtungsfleck*) of the zygote, they later appear on the anterior edge of the medullary plate or on the ectoderm adjacent to the plate. This indicates that the animal pole, which is conceived as lying within the germinal area, occurs on or very close to the boundary between presumptive epidermis and medullary material. This relationship is also shown in the following experiment.

In the four-cell stage a mark was placed on two cells at the intersection of the cleavage furrows (fig. 1). After the neural folds had met (fig. 2), the mark occurred on the anterior transverse fold, extending into the medullary plate on one side and into epidermis on the other. In later development at least part of the mark was on the epidermis at the tip of the head. The location of the mark near the close of neurulation (fig. 2) was very similar to the location assumed by marks made on the germinal area, that is, on the anterior edge of the medullary plate and on adjacent ectoderm. It follows, therefore, that the stain must have been placed close to the animal pole of the four-cell stage. Since the mark appeared on both epidermis and medullary plate, these presumptive materials must be contiguous in the region of the animal pole.

What is the destiny of marks placed on other parts of these micromeres?

In the eight-cell stage a single mark was placed on two micromeres not far from the point of intersection of the first two cleavage planes (fig. 3). When the neural folds became visible the mark was on ectoderm just outside of the medullary plate (fig. 4). In later development it occurred on the epidermis at the tip of the head, on the right eye, and on the lower portions of the mandibular arch and branchial area.

The stain in this case overlaps that made in the previous case, both appearing at the tip of the head; but the present mark, placed *farther* from the animal pole, extends *farther* into the epidermis. It thus appears that, as we leave the animal pole and move down one side of the egg, we are continually advancing more deeply into the territory of presumptive epidermis. How far can we thus advance before reaching its limits?

In the eight-cell stage of *Triturus torosus, no. 240,* a mark was placed on one micromere as far removed as possible from the animal pole (fig. 5). During gastrulation the mark continually approached closer to the blastopore; it then accompanied the neural folds as they converged dorsally, and was last observed adjacent to the posterior part of the right fold (fig. 6).

Since, as this experiment shows, marks made on micromeres as far removed as possible from the animal pole are still in the domain of presumptive epidermis, we must seek the boundary of this domain within the macromeres.

In the eight-cell stage of *Triturus torosus, no. 239,* mark *1* was made on two micromeres (fig. 7); while mark *2,* on the upper portion of a macromere, extends into the adjacent micromeres only to a slight extent.

When the neural folds (*nf.*, fig. 8) had met, mark *2* was on the ectoderm adjacent to the fold, while mark *1* was on the medullary plate, largely on its right margin.

The occurrence of mark *1*, the initial position of which was close to the animal pole, in the anterior portion of the medullary plate, indicates that presumptive medullary material must lie close to the region of the pole, and furthermore, that it occupies at least a substantial portion of certain micromeres. The experiment also indicates that the domain of presumptive epidermis reaches into the upper portions of one macromere.

Figure 9 shows an egg in the eight-cell stage, just after it was stained. Mark *1* was on portions of two micromeres; while mark *3* covered upper portions of two macromeres, encroaching to only a very slight extent upon one micromere. Mark *2* lay opposite to mark *3* but farther down on two macromeres.

During gastrulation all the marks stretched toward the blastopore; mark *2* entered it bodily, but only a small portion of mark *3* entered, most of it remaining on the external surface of the embryo after gastrulation was completed.

Figure 10 shows the embryo when the neural folds (*nf.*) had met. Mark *1* occurred on the fold and on the adjacent ectoderm. A portion of mark *3* was on the dorsal epidermis of the right side.

Again upper portions of some macromeres are seen to contain presumptive epidermis. But lower portions of these macromeres do not contain presumptive epidermis, as is evidenced by mark *2* which entered the blastopore and was therefore non-ectodermal.

The macromeral material stained in this experiment was on the right side of the egg; that stained in the previous experiment (figs. 7, 8) was on the left side. It follows that presumptive epidermis must occur in portions of at least two macromeres.

The area of presumptive epidermis delimited in the above experiments occurs on that side of the egg in which the pigment extends below the horizon, and which has been described by Daniel (1932) as the black (pigmented) shield.

The presumptive epidermis in both micromeres and macromeres is seen to stretch toward the blastopore. This stretching is more clearly illustrated in the following experiment.

B. THE PRESUMPTIVE EPIDERMIS DURING GASTRULATION, NEURULATION, AND HEAD FORMATION

A series of red and blue marks was placed around the circumference of a blastula, of which only the left side is shown in figure 11. If the embryo is observed as it appeared at the end of neurulation (fig. 14), it is seen that only marks *8* to *12* inclusive occurred partly or in their entirety on epidermis. We thus obtain clues as to the extent of the presumptive epidermis in figure 11. Its margin occurs between marks *7* and *8*.

In the U-shaped blastopore stage (fig. 12) the upper edges of the marks were still arranged in an approximately straight line as in the blastula stage. But when the blastopore (*bp.*, fig. 13) became slit-shaped, the upper ends of the marks were no longer thus arranged. Not only an elongation had occurred, but also a displacement of their anterior ends toward the blastopore. Lehmann (1929) has shown that the ectoderm is a region of pronounced cell division during gastrulation. The extent to which the movements here illustrated may be influenced by cell division is unknown.

In figure 14 marks *8* and *9* have been displaced dorsally and have elongated in a craniocaudal direction; mark *10* has elongated to a lesser extent. But marks *11* and *12* have elongated dorsalward rather than craniocaudally. Simultaneously the neural folds (*nf.*) have converged and the anterior end of the medullary plate has assumed a more ventral position. This complex of movements is analyzed into simpler components in the following experiments.

The medullary folds were not yet visible when a row of sixteen marks was placed on an embryo somewhat to the right of the midventral line (figs. 15, 16). The most posterior of these marks (*1–3*) lay adjacent to the blastopore (*bp.*). A second row of marks (*I–IV*) was placed on the dorsal side of the embryo.

In the racket-shaped medullary plate stage (figs. 17, 18) the circumblastoporal marks (*1–3*) have entered the blastopore, and are therefore no longer visible. Marks *4–12* have stretched in a dorsoventral direction. In contrast to this, marks *I–IV* have stretched in a craniocaudal direction.

After the appearance of the mandibular arch (fig. 19), mark *16* lay on the epidermis near the tip of the head, and on the mandibular arch.

Marks *15* and *14–13,* which had fused, were on the lower portions of the branchial area. Marks *4–12* spread fountain-like on the side of the body. The dorsal ends of marks *5–7,* formerly far anterior to the blastopore, now were posterior to the segment of the proctodeum (*pct.*). This change may only partially be accounted for by the flexure of the body. The dorsal ends of these marks actually rotate around the proctodeum as will be shown hereafter (see figs. 26, 27).

The ventral presumptive epidermis undergoes a pronounced dorsoventral elongation (stretching) during neurulation as is shown by marks *4–12* (figs. 15–19). But all parts of the ectoderm do not behave as does this ventral portion. Other modes of behavior are encountered when stains are placed, not near the midventral line, but along the lateral meridian.

In *Triturus torosus, no. 227,* a series of stains was made along the lateral meridian in the slit-shaped blastopore stage (fig. 20). During neurulation (fig. 21), marks *1–6* lay on the left margin of the medullary plate; marks *7–12* were on the neural fold and ectoderm adjacent to it. The marks (*1–7*) on the posterior part of the medullary plate have lengthened; the ectoderm adjacent to this part of the plate also lengthens, and in the same direction (see figs. 23–25). But marks on the ectoderm adjoining the *anterior* part of the plate (*8–12*) have not lengthened appreciably. The presumptive epidermis adjoining the entire medullary plate is thus divisible into two portions: a posterior portion which elongates craniocaudally during neurulation, and an anterior portion which does not.

Figure 22 shows the embryo when the mandibular arch (*md.*) was visible. Only marks *7–12* were seen externally. Marks *8–12* appeared on the epidermis covering the anterior part of the axial organs. A series of marks similar to those just described had been made on the opposite (right) side of the embryo; as on the left side, some were enclosed within the neural tube, while others came to occupy a position on the right side of the head similar to that occupied by marks *8–12* on the left side. We see, then, that a thin band of material which lies adjacent to the anterior part of the medullary plate forms the epidermis which covers the head.

This experiment gives us evidence concerning the epidermis of the mandibular arch, the eye, and the branchial region. The mandibular arch lay immediately posterior to mark *12;* the eye was in the posterior part of mark *11;* while the branchial area was adjacent to marks *8* and *9,* but did not extend up to mark *7.*

Since marks *1–6* were on the margin of the medullary plate, almost the entire dorsal half of the embryo shown in figure 20 must have been presumptive medullary material. This does not include a small circumblastoporal area which invaginates (see mark *1*). If a series of marks is placed slightly ventral to those made in this specimen, it may likewise be shown that the entire ventral half of the embryo, with the exception of a small circumblastoporal area, is presumptive epidermis.

An embryo so marked is shown in figure 23, the row of marks being somewhat more ventral than that seen in figure 20. During neurulation (fig. 24) marks *1–5* were on ectoderm adjacent to the medullary plate; marks *6–11* were somewhat removed from the plate.

When the mandibular arch (*md.*, fig. 25) appeared, marks *1–6* were on the epidermis covering that part of the axial organs posterior to the branchial region; marks *7–9* were elongated and extended obliquely forward and downward; mark *10* was just posterior to the branchial area. The dorsal part of the branchial area was covered by mark *7;* the ventral part by marks *8* and *9*. Mark *11* occurred on the opposite side of the embryo, covering the ventral portion of the branchial region.

It is striking that, although the stains were placed in the presumptive epidermis at the beginning of neurulation, we observe none of that dorsalward stretching seen in figures 14, 17, and 18. Instead of such a stretching, marks *1–6* have been carried bodily dorsalward, and while thus accompanying the converging neural folds have elongated in a craniocaudal direction.

Their behavior was similar to that seen in marks *1–6,* figures 20–22; these marks, it will be recalled, were on the medullary plate posterior to the branchial region and also elongated craniocaudally during convergence. Thus the ectoderm adjacent to the posterior part of the medullary plate, that part of the plate which forms most of the future spinal cord, behaves, not like the more ventral ectoderm, but rather as does the medullary material itself.

If we compare the behavior of marks *7* and *10* which indicate upper and lower portions of the branchial region respectively, we see that, while mark *7* has moved anteriorly and dorsally, mark *10* has moved posteriorly and ventrally. This wheeling movement changes the orientation of the presumptive epidermis of the head and branchial region. The craniocaudal orientation present at the beginning of neurulation, gives way to the approximate dorsoventral orientation of older embryos.

C. Movements of the Caudal Epidermis

In *Triturus torosus, no. 163,* two marks were made near the blastopore (fig. 26). Mark *1* was on the same level as the blastopore; mark *2* was ventrolateral to it. When the tail-bud became visible (fig. 27) mark *1* was posterior and dorsal to the proctodeum (*pct.*), while mark *2* extended dorsalward from it.

This experiment indicates that epidermal material lying immediately lateral and anterior to the blastopore during neurulation, comes to lie posterior and dorsal to the proctodeum, thus forming the lateral epidermis of the tail.

Figure 28 shows an embryo in which stains were placed close to the posterior part of the medullary plate. Marks *1* and *3* were on the left and right folds respectively, extending into the adjacent epidermis. Mark *2* was lateral and anterior to mark *1;* mark *4* was to the left of the blastopore.

In figure 29 the embryo is shown just before the closing of the neural folds. Marks *1* and *3* have converged. Mark *2* was now dorsal to mark *1.* Marks *1* and *4* were fused.

The relations of the marks to the developing tail are shown in figure 30. Marks *1* and *3* covered the ventral part of the tail-bud on the left and right sides respectively. Mark *2* included much of the dorsal epidermis on the left side. Dorsal and ventral epidermis of the tail therefore occur in the material adjacent to the posterior portion of the medullary plate. This material rotates around the future proctodeum during neurulation and formation of the tail-bud (*cf.* the position of marks *1* and *4* in figs. 29 and 30). Unfortunately the specimen was not observed further, so that the later development of the caudal epidermis must be illustrated by another embryo.

Figure 31 shows an embryo stained not long after the closing of the neural folds. With the appearance of the tail-bud (fig. 32) the more dorsal marks (*2* and *3*) had become elongated, while mark *1* had rotated downward. In later development (fig. 33), mark *1* appeared on the right side of the ventral fin, mark *3* on the right side of the dorsal fin, and mark *2* on the side of the tail.

We here observe the dorsal part of the tail elongating before the ventral part, in accordance with the pronounced stretching which occurs throughout the dorsal region of the embryo. By this unequal growth the early tail-bud comes to overhang the abdomen. Elongation of the ventral region begins somewhat later, resulting in equalization of dorsal and ventral portions of the tail.

D. Movements in the Epidermis Accompanying Reduction of Dorsal Flexure

In *Triturus torosus, no. 230,* a row of marks was placed on the ventral side of the embryo in the racket-shaped medullary plate stage (fig. 34). Fifteen hours later, when the mandibular arch was visible, most of the marks had stretched dorsalward (fig. 35). The elongation was not, however, so pronounced as that which occurred at the beginning of neurulation (see figs. 15–18).

In figure 36 we observe in marks *5* and *7* an elongation not seen since gastrulation, when the presumptive epidermis stretched toward the blastopore. Following gastrulation, that is, during neurulation, the ventral parts of the presumptive epidermis stretch *dorsalward.* But marks *5* and *7* in this specimen show that a craniocaudal stretching occurs again in the ventral epidermis after head and tail formation are well advanced. The dorsal flexure of the embryo, produced by rapid elongation in the dorsal region, is reduced by the growth of the ventral region. This is accompanied by stretching of the overlying epidermis.

SUMMARY

1. The movement and localization of the presumptive epidermis of *Triturus torosus* (Rathke), the common newt of the Pacific Coast of North America, have been studied by means of the Vogt method of vital staining.

2. Marks made on the germinal area (*Richtungsfleck*) appear on or adjacent to the anterior margin of the medullary plate.

3. Presumptive epidermis and medullary materials are contiguous in the region of the animal pole as early as the zygote stage.

4. A large portion, if not all, of two of the micromeres of the eight-cell stage contains presumptive epidermis; at least a large portion of two remaining micromeres is presumptive medullary material.

5. Presumptive epidermis occurs in parts of at least two macromeres adjacent to the epidermal micromeres.

6. The presumptive epidermis occurs on that side of the egg marked by the black (pigmented) shield.

7. The presumptive epidermis elongates (stretches) toward the blastopore during gastrulation.

8. Presumptive epidermis occupies approximately the ventral half of the embryo in the slit-shaped blastopore stage; presumptive medullary material occupies the remaining dorsal half (see p. 330).

9. Ventral portions of the presumptive epidermis, in the slit-shaped blastopore stage, stretch dorsalward during neurulation; dorsal portions, adjacent to the *posterior* part of the medullary material are carried dorsalward and simultaneously stretched in a craniocaudal direction, but dorsal portions, adjacent to the *anterior* part of the medullary plate undergo a wheeling movement (see p. 330).

10. The presumptive epidermis of the tail lies anterior to the blastopore during neurulation; during tail formation it rotates to positions posterior and dorsal to the proctodeum. The presumptive epidermis of the caudal fins lies adjacent to, and on either side of, the posterior portion of the medullary plate. The dorsal epidermis of the tail elongates first; later elongation of the ventral epidermis equalizes the extent of the epidermis in the dorsal and ventral fins.

11. The presumptive epidermis of the head is a narrow band adjacent to the anterior part of the medullary plate; that of the mandibular arch and the branchial area occupies narrow concentric bands around the presumptive epidermis of the head in the order indicated.

12. The dorsal flexure of the embryo is reduced by elongation in ventral parts; this is accompanied by corresponding elongations in the overlying epidermis.

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EXPLANATION OF PLATES

PLATE 33

Fig. 1. Location of stain (stippled) in four-cell stage of egg of *Triturus torosus, no. 209,* top view.

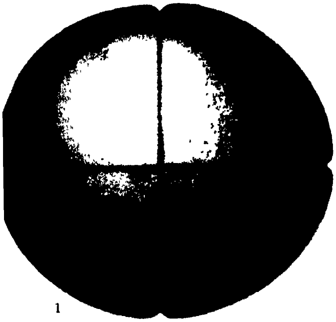
Fig. 2. Location of stain after the meeting of the neural folds (*nf.*) in the neurula of *Triturus torosus, no. 209,* anterior view.

Fig. 3. Location of stain (stippled) in eight-cell stage of egg of *Triturus torosus, no. 238,* top view.

Fig. 4. Location of stain in neurula of *Triturus torosus, no. 238, anterior view. nf.,* neural fold*; mp.,* medullary plate.

Fig. 5. Location of stain (stippled) in eight-cell stage of egg of *Triturus torosus, no. 240,* top view.

Fig. 6. Location of stain in the neurula of *Triturus torosus, no. 240,* posterior view.



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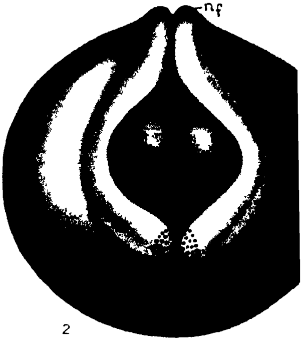

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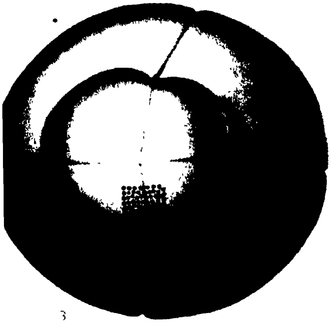

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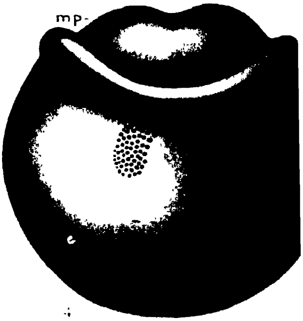

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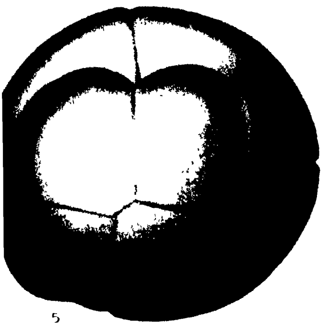

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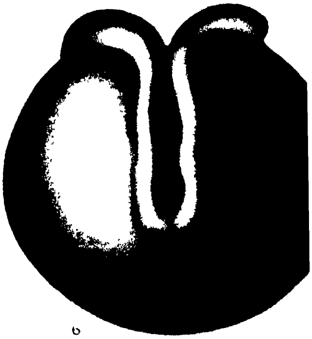

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PLATE 34

Fig. 7. Locations of stains (*1–2*) in eight-cell stage of egg of ***Triturus torosus, no. 239,*** top view.

Fig. 8. Locations of stains (*1–2*) after the meeting of the neural folds (*nf.*) in the neurula of ***Triturus torosus, no. 239,*** anterodorsal view.

Fig. 9. Locations of stains (*1–3*) in the eight-cell stage of egg of ***Triturus torosus, no. 242,*** top view.

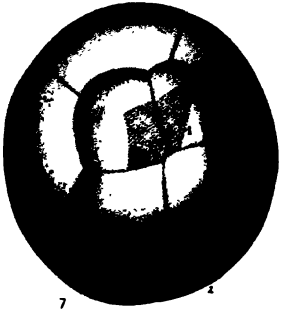
Fig. 10. Locations of stains after the meeting of the neural folds (*nf.*) in the neurula of ***Triturus torosus, no. 242,*** anterodorsal view.

Fig. 11. Locations of stains (*1–12*) in blastula of ***Triturus torosus, no. 229,*** view of the left side.

Fig. 12. Locations of stains (*1–12*) in the early gastrula of ***Triturus torosus, no. 229,*** view of the left side. ***bp., blastopore.***

Fig. 13. Locations of stains (*6–12*) in the late gastrula of ***Triturus torosus, no. 229,*** view of the left side. ***bp.,*** blastopore.

Fig. 14. Locations of stains (*8–12*) in the neurula of ***Triturus torosus, no. 229,*** view of the left side. ***nf.,*** neural fold.



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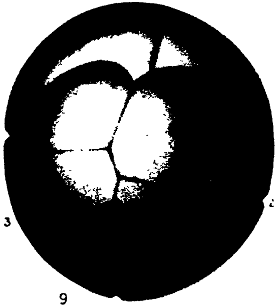

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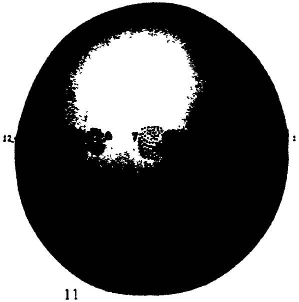

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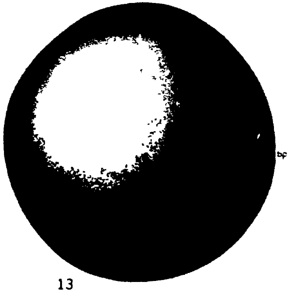

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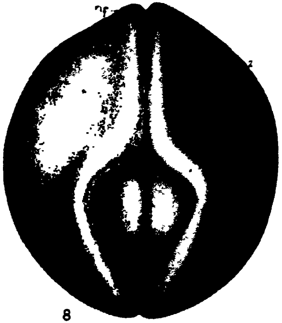

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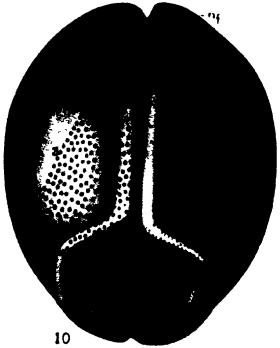

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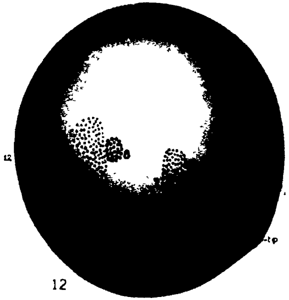

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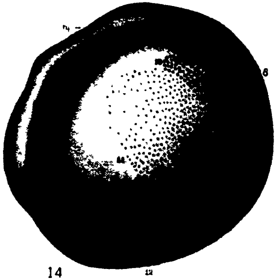

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PLATE 35

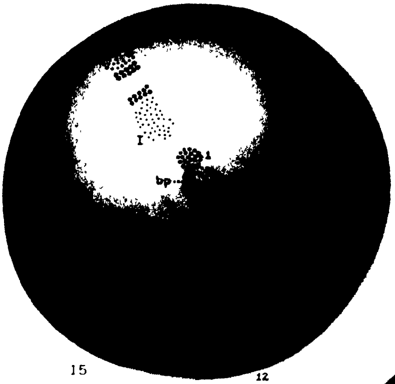
Fig. 15. Locations of stains (*I–IV* and *1–12*) in the late gastrula of *Triturus torosus, no. 228,* posterior view. *bp.,* blastopore.

Fig. 16. Locations of stains (*3–16*) in the late gastrula of *Triturus torosus, no. 228,* ventral view. *bp.,* blastopore.

Fig. 17. Locations of stains in the neurula of *Triturus torosus, no. 228, posterior* view. *mp.,* medullary plate; *nf.,* neural fold.

Fig. 18. Locations of stains (*4–16*) in the neurula of *Triturus torosus, no. 228,* ventral view. *mp.,* medullary plate; *nf.,* neural fold.

Fig. 19. Locations of stains (*4–16*) in advanced embryo of *Triturus torosus, no. 228,* ventral view. *pct.,* proctodeum.



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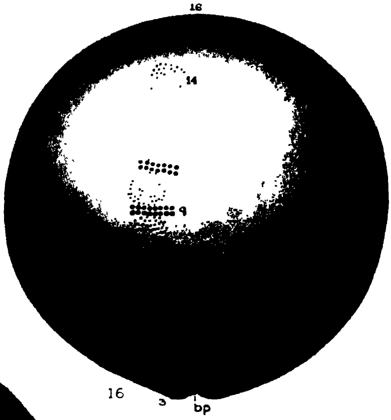

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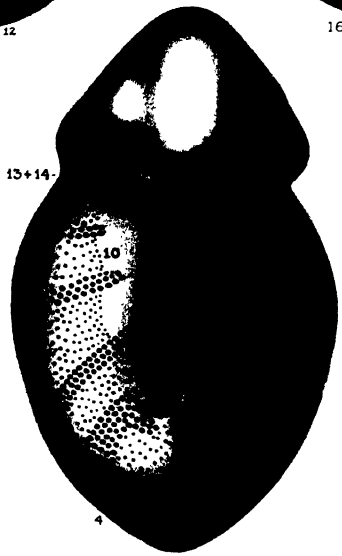

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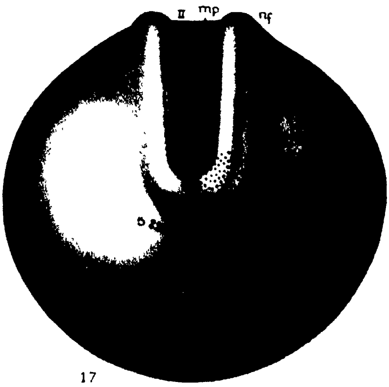

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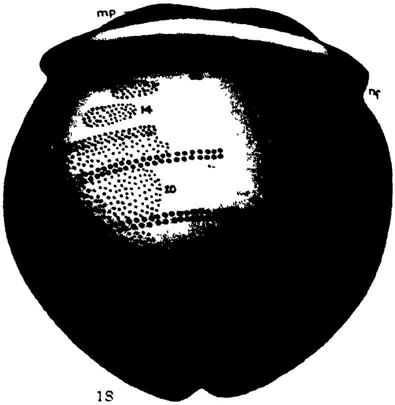

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PLATE 36

Fig. 20. Locations of stains (*1–12*) in late gastrula of *Triturus torosus, no. 227,* view of the left side. *bp.*, blastopore.

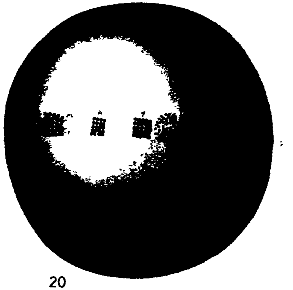
Fig. 21. Locations of stains (*1–12*) in the neurula of *Triturus torosus, no. 227,* view of the left side. *bp.*, blastopore; *mp.*, medullary plate.

Fig. 22. Locations of stains (*7–12*) in advanced embryo of *Triturus torosus, no. 227,* view of the left side. *md.*, mandibular arch.

Fig. 23. Locations of stains (*1–11*) in late gastrula of *Triturus torosus, no. 75,* view of the left side. *bp.*, blastopore.

Fig. 24. Locations of stains (*1–11*) in the neurula of *Triturus torosus, no. 75,* view of the left side. *mp.*, medullary plate; *nf.*, neural fold.

Fig. 25. Locations of stains (*1–10*) in advanced embryo of *Triturus torosus, no. 75,* view of the left side. *md.*, mandibular arch.



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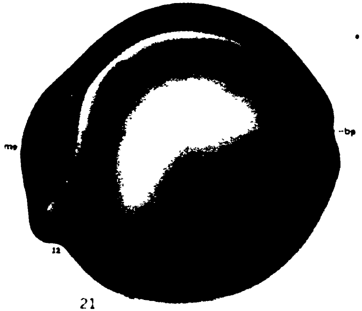

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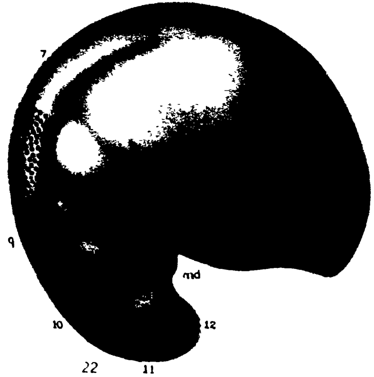

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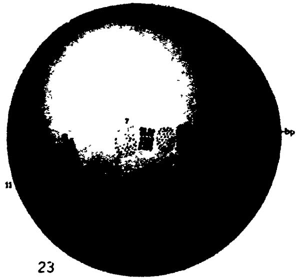

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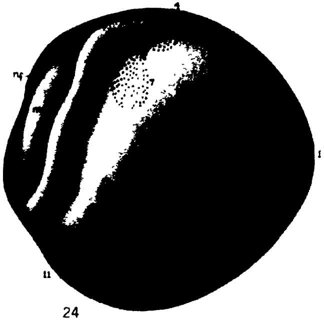

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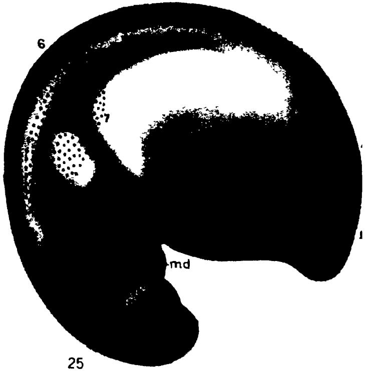

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PLATE 37

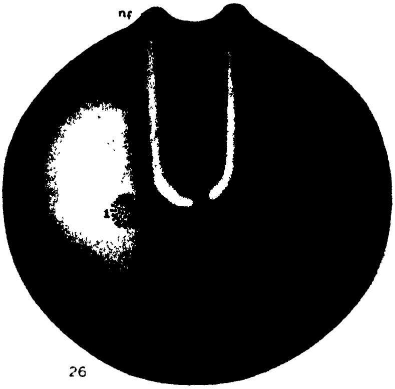
Fig. 26. Locations of stains (*1–2*) in neurula of *Triturus torosus, no. 163,* posterior view. *bp.*, blastopore.

Fig. 27. Locations of stains (*1–2*) in advanced embryo of *Triturus torosus, no. 163,* view of the right side. Stain *1*, on the left side, is shown as if the embryo were transparent.

Fig. 28. Locations of stains (*1–4*) in neurula of *Triturus torosus, no. 122,* posterior view. *bp.*, blastopore; *mp.*, medullary plate.

Fig. 29. Locations of stains (*1–4*) in neurula of *Triturus torosus, no. 122,* posterior view. *nf.*, neural fold.

Fig. 30. Locations of stains (*1–4*) in advanced embryo of *Triturus torosus, no. 122,* view of the left side. *pct.*, proctodeum.



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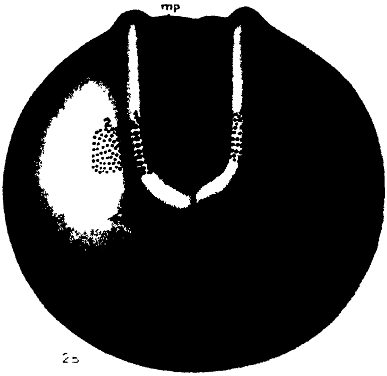

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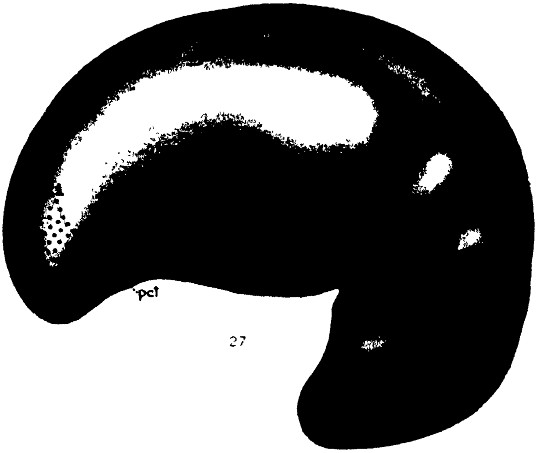

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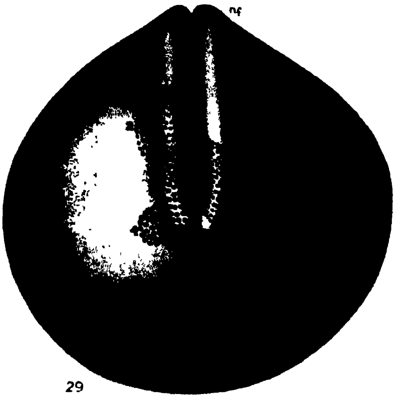

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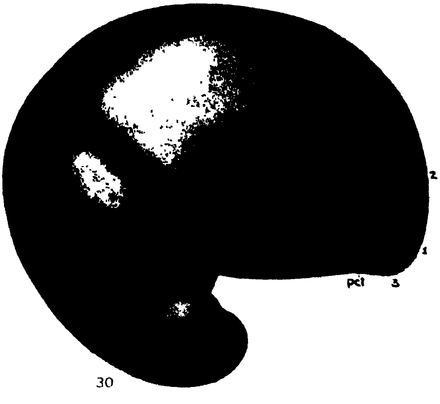

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PLATE 38

Fig. 31. Locations of stains (*1–3*) during early tail formation in embryo of *Triturus torosus, no. 35,* view of the right side.

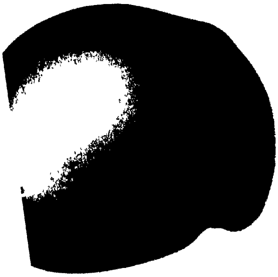
Fig. 32. Locations of stains (*1–3*) in a stage somewhat later than figure 31, *Triturus torosus, no. 35,* view of the right side.

Fig. 33. Locations of stains (*1–3*) in an advanced stage of tail formation, *Triturus torosus, no. 35,* view of the right side.

Fig. 34. Locations of stains (*1–11*) in late neurula of *Triturus torosus, no. 230,* ventral view. *bp.,* blastopore; *mp.,* medullary plate; *nf.,* neural fold.

Fig. 35. Locations of stains (*1–11*) during early head-formation in embryo of *Triturus torosus, no. 230,* ventral view. *pct.,* proctodeum.

Fig. 36. Locations of stains (*1, 3–11*) in advanced embryo of *Triturus torosus. no. 230,* ventral view. *pct.,* proctodeum.



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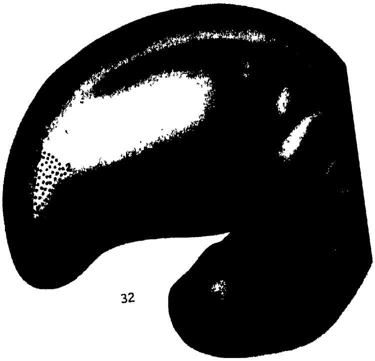

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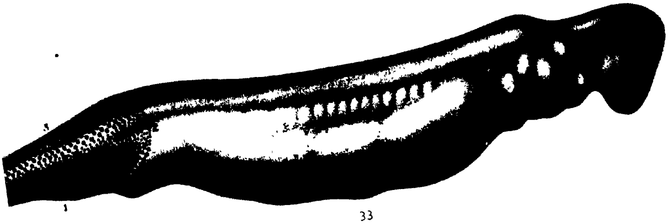

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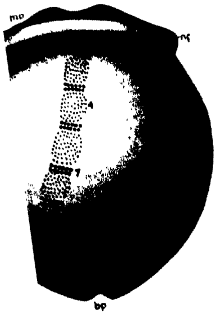

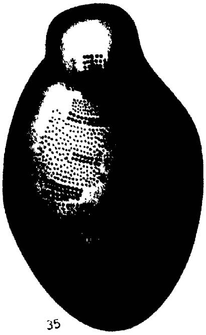

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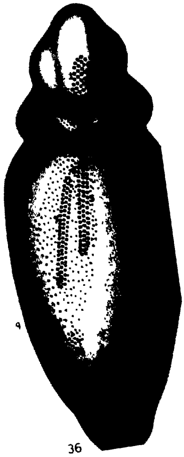

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ELECTROMETRIC pH DETERMINATIONS OF THE WALLS AND CONTENTS OF THE GASTRO-INTESTINAL TRACTS OF NORMAL ALBINO RATS

BY

CHARLES A. KOFOID, ETHEL McNEIL, AND RELDA CAILLEAU

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INTRODUCTION

Much work has been done on the pH of intestinal contents in various animals, but little of the work has been done on rodents. The determinations herein reported differ from most others in that the pH of the walls of the various parts of the digestive tract, as well as of the contents, has been determined.

These two determinations were thought to be a necessary preliminary to an attempt to discover whether or not the pH of the digestive tract, and in particular of the caecum, is related to the infectivity of the animal by pathogenic amoebae. Since such amoebae localize in the wall of the caecum in most experimental animals used, decided differences in acidity or alkalinity may be an important factor in influencing both the region of infection in the intestine and the percentage of infective animals.

The rat was used in the experiments primarily for three reasons: (1) it was available in large numbers; (2) it normally harbors species of the genera *Endamoeba* and *Councilmania* similar to those causing infection in man; and (3) it can be infected with the amoebae of the human digestive tract (Kessel, 1923).

EXPERIMENTAL

The rats were anesthetized by ether and were opened while still breathing strongly. The routine technique used was invariably carried out in the following manner. About two centimeters of the colon adjoining the caecum was removed, the contents pressed out for one determination, and the wall then scraped for the other determination. A 2-cm. segment of the ileum as it enters the caecum was similarly treated. Next, the caecum was opened and contents and wall scrapings removed. Then the duodenum was examined and determinations on the wall scrapings of the region nearest the stomach were made. Finally, the stomach was similarly treated, being first cut into two parts to separate the cardiac and pyloric portions, which are easily distinguishable in the rat.

The procedure in all cases was the same; each sample, whether contents or wall scrapings, was placed in a small glass dish and mixed with about 5 cc double distilled water. The materials were diluted to approximately the same volume in each test. The amount of the intestinal contents or scrapings varied greatly, yet no changes in pH values were discernible even when the dilution with water was increased ten times. It was therefore thought to be more expedient to dilute to a constant volume rather than to attempt to approximate the same amount of material each time. The quinhydrone reference electrode was used.

The entire process consumed less than 15 minutes from the time the rat was opened until the final reading was made. The rat was still living when the final portions were removed and the tissues were as near the normal condition as possible. One worker removed the tissues and contents while the other worker immediately made the determinations in the order in which the portions were removed. In this way a minimum of time elapsed between the time the tissues were removed from the living animal and the determination of the pH. In all cases the material to be tested was never allowed to stand over 5 minutes, at the most, before readings were made.

Attempts were made to get rats in a starving condition, or in the post-absorptive state, as it was thought that more accurate or reliable results of the pH of the walls could be obtained in this way. However, only when the animal had been starved 72 hours could the digestive tract as a whole be considered relatively empty, contents otherwise always being found in both caecum and stomach. The rats under these conditions usually showed signs of inflammation or fatty degeneration all along the tract. Best results were obtained by removing the rats from the cages containing food about three hours after they had been fed, and keeping them in a cage containing water only, until the fol-

Ten female albino rats were used for one of the series, all the other results were completed on male albino rats. No significant differences between the sexes could be found. All the animals used were adult rats about nine months old.

Determinations were made upon the following regions:

1. Wall and contents of the cardiac portion of the stomach nearest the esophagus
2. Wall and contents of the pyloric portion of the stomach nearest the duodenum
3. Wall scrapings of the duodenum (contents were never available) nearest the stomach
4. Wall and contents of about 2 cm. of the ileum as it enters the caecum
5. Wall and contents of the caecum
6. Wall and contents of the colon as it leaves the caecum
7. Faeces

After the results had been recorded, a survey of the type diet used was made in order to check with the results of other workers. The ration was apparently of the "low fat" type diet although not quite so low as that of Zucker (1924), which had a fat content of less than 3 per cent. It was not a rachitogenic diet, as it contained sufficient vitamin D.

COMPOSITION OF RAT MAINTENANCE DIET

	Per cent
Ground wheat	67.5
Alfalfa meal 20 per cent protein	10.5
Fish meal	10.5
Fish oil Sardeline (source of vitamin D)	5.25
Casein	5.25
Salt	1.0

The food itself had a pH of 5.5.

DISCUSSION OF RESULTS

Very probably the relatively low-fat diet of our rats was responsible for the divergencies of our results, which proved to be considerably higher than those of other workers. Redman, Willimott, and Wokes (1927), working on normal rats and on rats fed on rachitogenic diets with low or high-fat content, obtained the following averages for their normal animals: stomach 4.0, duodenum 5.5, jejunum 6.0, ileum 6.8, caecum and large intestine 6.5. Their rats fed on Zucker's "low fat" rachitogenic diet developed an alkaline reaction throughout the intestinal tract, the caecum and the large intestine averaging pH 7.3.

Similar results were obtained by Abrahamson and Miller (1925) by colorimetric means. Their analyses showed the averages to be: stomach 3.8, upper half of the intestine 5.8, lower half of the intestine 6.0. On a rachitic diet the rats showed an increased alkalinity in the intestine, the average being pH 7.0. Graysel and Miller (1925), working on dogs, obtained results practically identical with those of the above workers, and Zucker and Matzner (1924) found that the faeces of rats fed on a rickets-producing diet had a pH of 7.6 but that the addition of cod liver oil reduced the pH to 6.2 or lower. All these workers based their results on contents only and did not consider the pH of the walls.

Our averages were as follows: cardiac contents 3.95, cardiac wall 4.58, pyloric contents 3.34, pyloric wall 4.71, duodenum wall 6.93, ileum contents 7.51, ileum wall 6.98, caecal contents 7.13, caecal wall 7.34, colon contents 7.33, colon wall 6.95, faeces 7.14.

Since our results differ considerably from those of other workers, a word of explanation is necessary. As ours seems to be one of the few attempts to utilize both wall and contents in making the determinations, diverging results are to be expected, although our readings obtained on stomach contents agree generally with other published results. Our work shows that the contents become increasingly more acid from the cardiac to the pyloric region of the stomach, whereas the stomach walls show a tendency toward the opposite reaction in the same region. This appears natural when one remembers that the contents of the pyloric portion have undergone longer exposure to the gastric juices, and that the pyloric wall of the stomach is less acid than the cardiac wall, since there is little if any secretion of hydrochloric acid in that region.

The duodenal wall appears to be practically neutral in reaction (6.93), and the consistent readings cannot be overlooked; for although our readings appear much higher than previously reported results, the other workers have reported on duodenal *contents* only. Their lower readings were merely indicative of the acid contents from the stomach being made more alkaline by the pancreatic and other enzymes, and gave no indication of the actual pH of the wall.

The pH of the *wall* of the lower ileum is about neutral. Other workers have reported a pH of 6.8 for the ileum content; our results give an average of 7.51. This difference is probably owing to the fact that they used a diet of higher fat content; therefore more fatty acids were formed and the acidity increased.

The wall of the caecum is slightly alkaline (7.35). We obtained a pH of 7.15 for the contents, which bears out the fact that the caecum of herbiverous animals, unlike that of carnivorous animals, neutralizes the acids formed by fermentation and causes the reaction to remain practically neutral.

CHART 1

Animal	Condition	Stomach	Duodenum	Ileum	Caecum	Colon	Faeces
1	Fed 20 hours before		6 84	6.00	7.60	7.60	
2	Fed 20 hours before	2.76	6.75	7.34	7.51	7.87	7.17
3	Starved 24 hours		6.92	7.26	7.43	7.60	7.17
4	Starved 24 hours, diarrheic		7.17		7.68	7.01	7.68
5	Starved 24 hours	3.36		7.60	7.51	7.60	7.68
6	Starved 24 hours	3.23		7.17	7.50	7.57	7.34
7	Fed large meal 48 hours before	3.79	7.01		7.09	7.09	7.68
8	Fed large meal 48 hours before	3.25	7.09	7.51	6.43	6.92	7.09
9	Fed large meal 48 hours before	2.45	6.92	7.09	6.33	7.17	6.33
10	Fed large meal 48 hours before	2.53	7.26	7.51	6.33	7.17	6 33

CHART 2

Animal: Rat	Condition	Cardiac Stomach		Pyloric stomach		Duodenum Wall	Ileum		Caecum		Colon		Faeces
		Contents	Wall	Contents	Wall		Contents	Wall	Contents	Wall	Contents	Wall	
11	Starved 72 hours	3.87	3.71	3.34	4.21	5.92	6.60	6.78	7.17	7.43		6.10	
12	Starved 72 hours	2.68	3.52	2.80	4.55	6.25	7.15	6.33	7.46	7.38		6.33	
13	1½ hours after feeding	5.06	5.23		2.55	6.16	7.15	6.61	7.63	7.48	7.69	7.64	7.43
14	4 hours after feeding	4.55	4.71	3.23	3.96	7.60	7.43	7.52	7.20	7.52	7.65	7.26	6.84
15	5 hours after feeding	4.63	4.80	3.05	3.71	7.12	7.26	7.26	6.75	7.17		7.43	
16	12 hours after feeding	3.36	4.71	3.23	3.52	6.75	7.09	6.60	6.42	7.01	6.52	7.60	6.68
17	12 hours after feeding			2.76	3.49	7.09	7.34	7.01	6.84	7.01	7.09	7.17	7.01
18	24 hours after large meal	5.30	4.63	3.71	4.88	5 92	7.52	7.26	6.60	6.92	7.20	6.84	6.92
19	24 hours after large meal, tract inflamed	3.98	4.29	3.03	3.98	6.92	7.75	7.34	7.01	7.26	7.52	6.92	7.26
20	24 hours after large meal, tract normal	4.63	5.06	3.20	3.98	6.84	7.52	6.92	6.60	7.09	6.92	6.68	6.92
21	Fed 24 hours before. Digestive tract full	3.96	4.21	3.71	5.55	6.92	7.52	6.92	6.84	7.01	6.53	6.84	6.60
22	Fed 24 hours before. Digestive tract full	4.38	4.55	3.63	5.06	6.92	8.03	7.09	7.01	7.26	6.92	7.01	7.17
23	Fed 24 hours before. Stomach nearly empty	3.03	3.71	3.20	4.63	6.84	7.68	7.01	6.84	7.17	7.09	7.01	6.92
24	Starved 72 hours. Digestive tract nearly empty and inflamed	3.52	4.97	4.80	5.91	7.01	8.00	6.84	7.92	8.17	7.77	7.09	7.43
25	Starved 72 hours. Digestive tract nearly empty and inflamed	3.11	4.12	4.29	6.53	7.26	8.00	7.09	8.09	7.76	7.86	7.26	8.08
27	Starved 72 hours, tract normal	3.71	4.21	3.20	4.55	7.09	7.86	7.09	7.77	7.52	8.00	7.09	7.34
28	Starved 36 hours. Stomach nearly empty	4.12	4.80	3.36	5.23	7.01	8.00	7.17	7.09	7.26	7.95	7.17	
29	Starved 36 hours. Stomach nearly empty	3.36	4.55	3.87	5.83	7.09	8.00	7.26	6.26	7.76	7.68	6.84	7.34
30	Starved 36 hours. Stomach nearly empty	3.71	5.23	4.47	6.25	7.17		6.68	7.43	7.43	6.26	6.60	6.33

CHART 3

Animal: Rat	Condition	Cardiac Stomach		Pyloric stomach		Duodenum Wall	Ileum		Caecum		Colon		Faeces
		Contents	Wall	Contents	Wall		Contents	Wall	Contents	Wall	Contents	Wall	
31	Fed 24 hours before, stomach empty	4.38	5.38	3.63	4.88	7.01	7.85	7.01	7.26	7.43	7.43	6.92	7.43
32	Fed 24 hours before, stomach empty	3.52	4.29	3.20	4.80	7.09	7.77	7.09	6.92	7.43	7.26	6.92	7.34
33	Fed 24 hours before, stomach full	4.88	5.46	3.35	4.71	7.09	7.68	7.09	6.75	7.26	7.34	6.92	7.26
34	Fed 24 hours before, stomach nearly empty..	3.79	4.38	3.10	4.13	7.09	7.77	7.01	6.92	7.52	7.17	6.84	7.09
35	Fed 24 hours before, stomach full .	3.87	4.71	2.57	4.55	7.09	7.68	7.09	6.92	7.34	7.26	6.92	7.43
36	Fed 24 hours before, stomach nearly empty..	4.21	4.55	3.27	5.15	7.05	7.85	7.17	7.09	7.34	6.84	7.09	7.26
37	Fed 24 hours before, caecum hemorrhagic, colon gaseous	3.13	4.80	3.20	4.96	7.09	7.52	6.84	7.43	7.09	6.68	6.92	7.17
38	Fed 24 hours before, stomach nearly empty..	4.29	4.88	4.13	5.82	7.09	7.92	7.09	7.26	7.52	7.52	7.09	7.17
39	Fed 24 hours before, stomach full...	5.30	5.73	3.44	4.71	7.01	7.77	7.01	6.75	7.17	7.01	7.09	6.92
40	Fed 24 hours before, stomach nearly empty..	3.10	3.87	2.76	4.21	6.68	7.92	6.53	6.42	7.01	7.17	6.60	7.01

AVERAGES

Cardiac contents	Cardiac wall	Pyloric contents	Pyloric wall	Duodenum wall	Ileum contents	Ileum wall	Caecum contents	Caecum wall	Colon contents	Colon wall	Faeces
29 determinations	29 reading	36 rats	30 rats	38 rats	37 rats	30 rats	40 rats	30 rats	36 rats	30 rats	34 rats
3.95	4.58	3.34	4.71	6.93	7.51	6.98	7.13	7.34	7.33	6.95	7.14

SUMMARY

An electrometric method, using the quinhydrone electrode, was used on albino rats to determine the pH of the wall and of the contents of the entire gastro-intestinal tract.

A relatively low-fat diet was used on normal albino rats and yielded the following average pH values: cardiac contents 3.95, cardiac wall 4.58, pyloric contents 3.34, pyloric wall 4.71, duodenum wall 6.93, ileum contents 7.51, ileum wall 6.98, caecum contents 7.13, caecum wall 7.34, colon contents 7.33, colon wall 6.95, faeces 7.14.

The rats tested were not examined for intestinal amoebae, but other rats living under identical conditions and in the same cages showed a great number of amoebae and flagellates in the caecum, indicating that a neutral or alkaline range, approximately the pH of the blood, is optimum for the growth of these Protozoa of the rat.

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A PRELIMINARY STUDY OF THE NITROGEN NEEDS OF GROWING TERMOPSIS

BY

ELIZABETH S. ROESSLER

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Termites present interesting metabolic problems. Although few animals can utilize cellulose and no animals are known to be able to live without proteins, laboratory colonies of certain termites seem to do these very things. The genus *Termopsis*, particularly the two common species, *T. angusticollis* and *T. nevadensis,* appear to live indefinitely, if properly cared for, on a diet of filter paper and water, with no apparent source of nitrogen or other elements necessary to life, such as sulphur and phosphorous. This has been the general experience of investigators who have kept these animals in laboratory cultures for various purposes.

Cleveland (1925) maintains, as a result of experiment, not only that pure cellulose is as nutritious as a normal diet of wood but that on it as a sole diet termites can maintain themselves indefinitely and continue normal growth. Therefore, he concludes, they "must be able in some way to fix atmospheric nitrogen, which they use in manufacturing proteins; or else, contrary to current opinion, they must be able to transform carbohydrates into proteins." It seems highly probable that Cleveland's methods were at fault or that his colonies drew on nitrogen reserves which were nearing their limit. The first step, therefore, in a search for the source of the nitrogen supply of termites, has been to determine whether termites really thrive on pure cellulose.

Grateful acknowledgment is made to Dr. S. F. Light for his stimulating interest and criticism and to Dr. S. F. Cook for many helpful suggestions. The author is indebted to Dr. T. D. Beckwith for assistance in supplying the culture media for growing fungus.

MATERIAL, METHODS, AND RESULTS

All termites used were *Termopsis nevadensis* Hagen, collected by the writer at Mount Hermon, California. Whatman's filter paper No. 44 was used as the cellulose diet. Of course, this "pure cellulose," as it is called below, was only relatively pure; it was undoubtedly the seat of growth of both bacteria and fungi, as completely sterile cultures were out of the question. The decayed wood was taken from the Douglas fir stumps in which the experimental animals had been found. The sound wood used was Douglas fir. Enough distilled water to keep the food moist was dropped daily onto the paper and wood; approximately the same number of drops of water was given to each group each day. The termites were kept in covered finger bowls, in the dark, and handled as little and as gently as possible. Each group of animals was weighed as a unit to the accuracy of one one-thousandth of a gram; the average weights per termite were carried to the fourth place, with a probable error of .0005.

The first series of experiments was designed to detect a possible difference in the rate of growth between the termites fed on pure filter paper and those fed on the normal diet of decayed wood which had been cleaned of pellets and débris. Seven groups, each consisting of 50 termites, 25 first-form reproductive nymphs and 25 soldier nymphs, all of the sixth instar, were set up and fed as follows:

A1: Filter paper; paper and dish changed daily to eliminate extensive use of pellets and fungus as food.
B1: Decayed wood; wood and dish changed daily for same reason.
C1: Filter paper; not changed.
D1: Wood; not changed.
E1: Filter paper; paper suspended above fine copper screening and changed daily; the pellets fell through the screening and hence could not be eaten.
F1: Filter paper; paper suspended above screening but not changed.
G1: Decayed wood; wood above screening and changed daily.

In C1, D1, and F1 pellets and débris were allowed to remain in order to test any possible effect on the growth rate and also as a control check against the daily changing of the other groups. The termites were transferred to a clean dish and onto clean food by allowing them to run of their own accord from the old piece of paper, or wood, to the new. By taking advantage of their strong negative phototropism this was accomplished rather quickly and without the necessity of touching them. An effort was made to remove dead termites before they could be eaten by the others, but this was not successful, since termites which died during the night were usually devoured wholly or in part (see table 1).

A week later, duplicates of C1 and D1 were set up and called C2 and D2. Two weeks later seven similarly treated groups, this time of 100 animals each, were set up and labeled A3 to G3, respectively.

After a little over two months, all 16 groups were again weighed and the results compiled in table 1.

The number of deaths varied with each group and seemed to be due to the small size of the groups and to have no significant relation to the diet. Similarly, the development of alates, which unfortunately, had to be removed, seemed to bear no relation to the diet, as the figures indicate.

The consistency of the figures for the increase in weight per termite seemed important. In every case the termites feeding on wood gained more than those feeding on filter paper under similar treatment. Also, with the exception of the first four groups, A1, B1, C1, and D1, for which the preliminary weighings were made on inaccurate scales, the animals on the wood diet now weighed more per individual than those similarly treated on the filter paper diet, regardless of initial weights (see fig. 1). Some of the colonies feeding on filter paper actually lost weight, notably C3 in which only two dead members served as food, and F1 and E3 in which the filter paper was suspended over screening, thus eliminating pellets. The weight increase of each group as a whole was calculated as if none had died or developed wings and this estimated weight for each group feeding on wood was more than that for the corresponding group feeding on filter paper even though some of the filter paper colonies weighed more at the start.

In spite of the many sources of error in these figures, the changes in weight are consistent with the differences in food and with these differences only. In spite of inaccuracies, therefore, these first results showed a need on the part of *Termopsis* for nutriment not to be found in pure cellulose.

In order more thoroughly to prove the contention that a cellulose diet lacks some quality necessary to vigorous growth which wood, or something closely associated with wood, supplies, all animals previously kept on a filter paper diet (except A3 and C3, see below) were

TABLE 1

Experiment	A1	B1	C1	D1	C2	D2	E1	F1	G1	A3	B3	C3	D3	E3	F3	G3
Food	filter paper	wood	filter paper	wood	filter paper	wood	filter paper	filter paper	wood	filter paper	wood	filter paper	wood	filter paper	filter paper	wood
Average weight in grams at beginning	.0474	.0428	.0429	.0418	.0360	.0380	.0426	.0423	.0403	.0346	.0339	.0374	.0308	.0360	.0351	.0376
Average weight in grams after 2 months	.0481	.0470	.0485	.0499	.0384	.0465	.0427	.0420	.0470	.0368	.0418	.0364	.0384	.0344	.0351	.0405
Difference	+.0007	+.0042	+.0056	+.0081	+.0024	+.0085	+.0001	-.0003	+.0067	+.0022	+.0079	-.0010	+.0076	-.0016	.0000	+.0029
Number in original set up	50	50	50	50	50	50	50	50	50	100	100	100	100	100	100	100
Number of alates developed and removed	8	2	9	11	4	11	4	4	3	3	1	2	7	7	19	2
Number died and eaten by others	9	9	12	13	6	11	7	7	15	13	10	2	20	14	14	?

put together and given a diet of wood; all those previously kept on a wood diet (except B3 and D3) were put together and given a filter paper diet. A month later the average weight per termite of those transferred from filter paper to wood had increased from .0398 grams to .0430 grams, while the average weight of those changed from wood to filter paper had dropped from .0458 grams to .0417 grams.

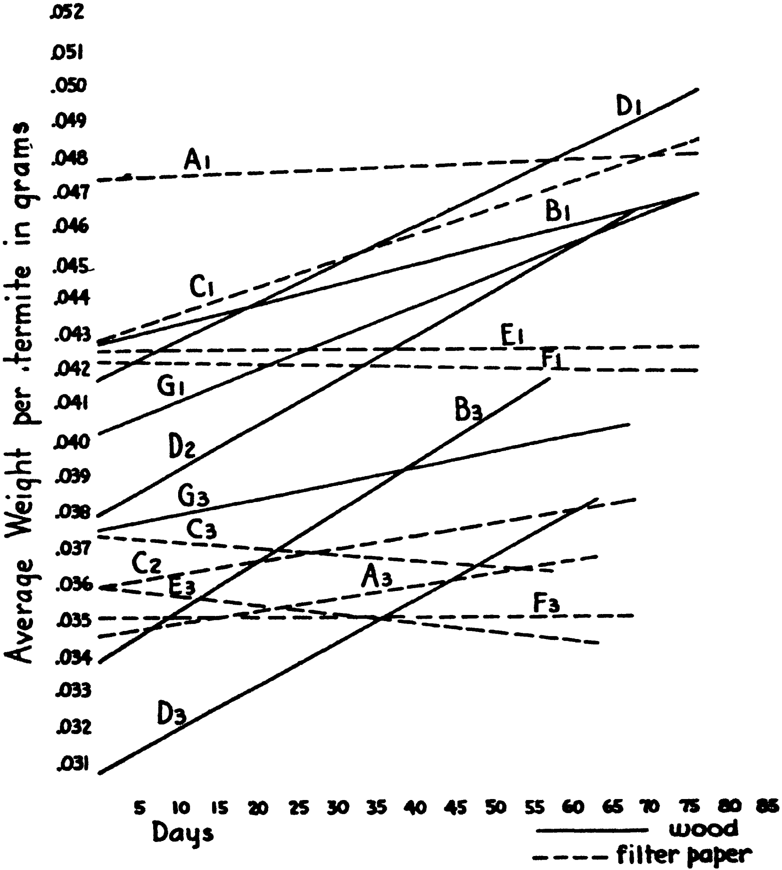

Fig. 1

Termopsis lives but does not grow well on pure (or almost pure) cellulose. Will it continue to live indefinitely on it? To answer this question, groups A3, B3 (filter paper and wood, respectively, changed daily), and C3 and D3 (filter paper and wood, respectively, not changed) were continued. After 225 days, all groups had gained weight but those feeding on wood were heavier, having gained more rapidly, and fewer of these had died (see table 2). There was no significant dying out of those feeding on filter paper, possibly because the "pure cellulose" was not pure enough, possibly because the experiment was not continued long enough—probably because of both reasons.

As growth might prove to be more rapid in the early instars and hence differences due to diet be more pronounced, two groups of very small termites were started: A4 on filter paper and D4 on decayed wood. Each group consisted of 20 first and second instar and 164 third and fourth instar individuals. Twenty nymphs of the sixth instar were put with each colony to take care of the little ones; these sixth instar nymphs were not included in the weighing and were removed when no longer needed. Two groups, each composed of 200 fifth instar nymphs, were set up, A5 on filter paper and D5 on wood. In all these colonies the animals were sibs. No alates developed. The water was carefully administered; the same number of drops was given to each group. Cannibalism, unfortunately, proved to be unavoidable. Weighings were made at monthly intervals.

TABLE 2

	A3	B3	C3	D3
Food	filter paper	wood	filter paper	wood
Average weight in grams at beginning	.0346	.0339	.0374	.0308
Average weight in grams after 225 days	.0451	.0524	.0466	.0516
Difference	+.0105	+.0185	+.0092	+.0208
Number, out of 100 that started, alive at end	28	41	31	32

At the end of five months, the gains made were as follows:

TABLE 3

	A4	D4	A5	D5
Food	filter paper	wood	filter paper	wood
Average weight in grams at beginning	.0073	.0071	.0137	.0141
Average weight in grams at end of five months	.0180	.0226	.0234	.0303
Difference	+.0107	+.0155	+.0097	+.0162

The growth curve is approximately a straight line, and in both cases the curve representing the group on the wood diet steadily diverges from the one for the filter paper group (see fig. 2).

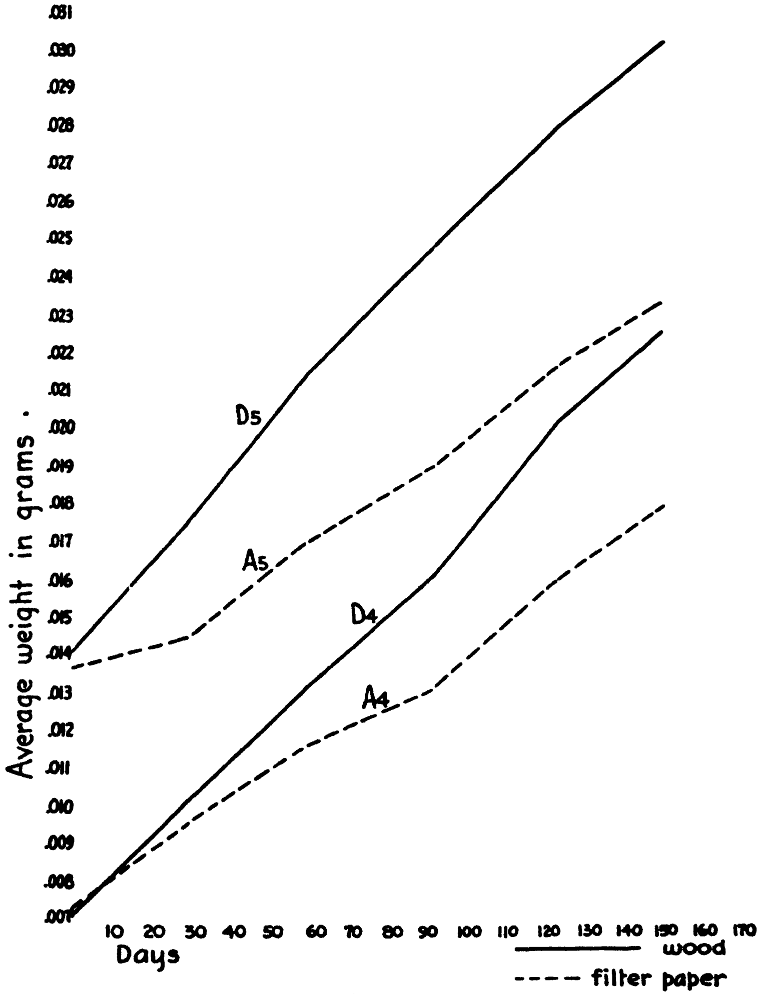

Fig. 2

The most surprising and interesting results of this particular experiment were those pertaining to the development of wing pads. Table 4 (p. 364) shows the number of termites on the respective foods, that had developed wing pads at the end of five months.

The animals were sibs, selected at random. The A5 group, in which only four developed wing pads, was composed of larger, heavier animals than those in D4, half of which developed wing pads; therefore such development is not explainable merely by size. The figures seem quite certainly to indicate that the development of wing pads is inhibited by lack of certain foods. They might be construed to imply that the development of first forms, and hence of other castes, is conditioned by the diet; but the development of wing pads is not necessarily caste development, therefore any general conclusions in this regard would be untimely.

TABLE 4

	A4	D4	A5	D5
Food	filter paper	wood	filter paper	wood
Number of termites living at end of experiment	72	106	78	108
Number with wing pads	0	51	4	88

With this caste problem in mind, as well as the original problem, six groups of supplementary reproductives together with early sixth instar soldier nymphs were set up, three colonies on wood and three on filter paper. At the end of five months there were large numbers of small termites in all six dishes, no difference in reproductive capacity being detected between those eating wood and those eating cellulose. Unfortunately, in the writer's absence, all these colonies were accidentally destroyed before any data were available regarding the castes of the new members or the relative growth rates.

Assuming that termites gain more when feeding on decayed wood than on filter paper, it probably follows that they need, besides cellulose, nitrogen and other elements such as sulphur and phosphorous. Do they get these elements from the wood itself, from fungi growing in decayed wood, or from something else, such as nitrates, associated with the decay of wood?

Soluble alkali nitrates are formed in nature through the decomposition of organic material. With the thought that termites, by means of microorganisms of one kind or another, might possibly utilize nitrogen in this form, five groups of 100 animals each were fed filter paper moistened respectively with 1-1000 molal solutions of potassium nitrate, calcium nitrate, sodium nitrate, ammonium nitrate, and pure water. Over a period of three months, three of the four groups on the nitrates gained more than the one on pure water; the ones on calcium nitrate and ammonium nitrate at first lagged and later gained the most rapidly. One set of figures is, however, not very significant and none of these made any striking gains (see table 5).

TABLE 5

Food	H_2O filter paper	$Ca(NO_3)_2$ filter paper	NH_4NO_3 filter paper	KNO_3 filter paper	$NaNO_3$ filter paper
Original average weight in grams	.0205	.0204	.0203	.0205	.0211
Average weight after one month	.0206	.0211	.0205	.0225	.0225
Average weight after two months	.0217	.0224	.0224	.0250	.0234
Average weight after three months	.0242	.0270	.0261	.0254	.0247

In a further effort to determine the source of these nutritive elements lacking in cellulose, four groups of 100 animals each were set up and fed as follows:

W: Decayed Douglas fir wood.
X: Sound Douglas fir wood, sterilized in an autoclave and changed every other day in order to retard fungus growth.
Y: Filter paper dampened with sterile aqueous extract of Douglas fir wood, which would contain all the water soluble substances of the wood.
Z: Filter paper and pure water.

The termites on sound, sterile wood gained weight faster than those on decayed wood, and those on wood extract gained more than the ones on pure water; but the casualties were extraordinarily high in this experiment (see table 6) and prevent the drawing of any definite conclusions.

TABLE 6

	W	X	Y	Z
Food	decayed wood	sound wood	wood extract filter paper	H_2O and filter paper
Original average weight in grams	.0173	.0173	.0166	.0166
Average weight after 30 days	.0192	.0202	.0179	.0173
Average weight after 70 days	.0207	.0230	.0211	.0209
Average weight after 124 days	.0230	.0270	.0280	.0262
Number, out of 100 that started, alive after 124 days	72	59	26	37

Fungi, which termites eat, were considered as a possible source of nitrogen and other important elements. Cultures were made from the guts of termites, held in iodine for one minute to sterilize them on the outside. The most prevalent fungus in all the *Termopsis* opened was a kind of Mucorales. This was grown on plates of agar and malt extract and fed to two groups of 100 termites each, one kept on filter paper and the other on sterile wood, with controls on filter paper and sterile wood without fungus. During ten weeks, the control group in each case gained just a trifle more than the group with fungus. Apparently this species of fungus, seemingly the one most eaten by *Termopsis*, has no special food value. It is significant to note that the growth curves for the two groups fed on sterile wood, both with and without fungus, diverged steadily from the two groups fed on filter paper (see fig. 3).

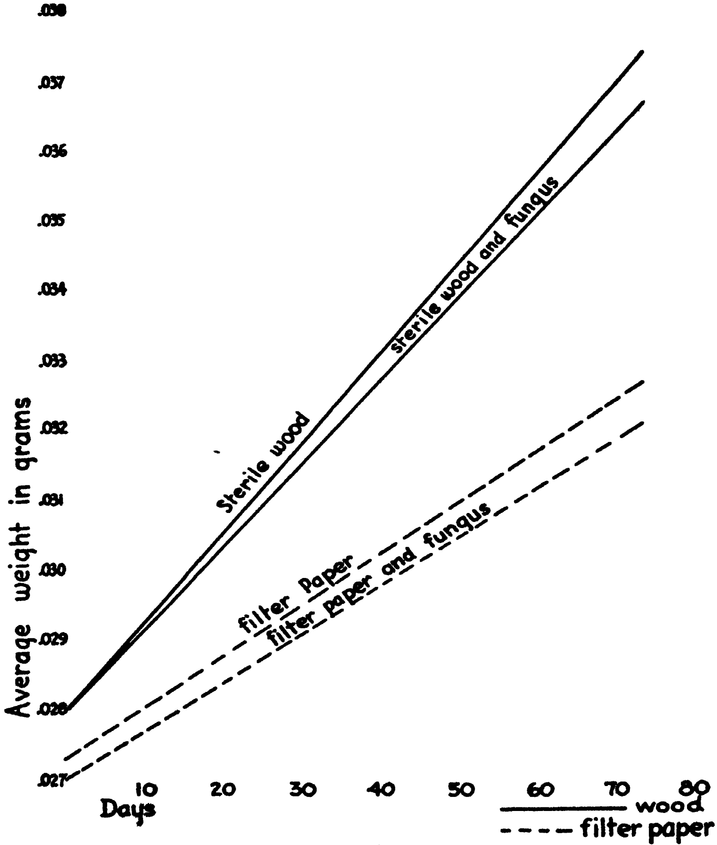

Fig. 3

It seemed possible that the entire set of experiments might be invalidated by the ever present cannibalism. Perhaps the termites got enough food material from their dead fellows, regardless of their imposed diet, to live longer than any experiments had been run. Therefore it was decided to keep four diet groups with each individual in a separate vial. Four hundred termites were put in as many tubes. The first hundred were given sterile sound wood; the second hundred, decayed wood; the third hundred, filter paper; and the fourth hundred, filter paper moistened with sterile wood extract instead of water. As a control group a fifth hundred was kept together and on filter paper. The animals were weighed as groups before and after the experiment. About one-fourth of the isolated termites had died at the end of six weeks. The ones on sound wood gained most (see fig. 4) and those on wood extract came next. All of them gained except those on the decayed wood diet, which remained at the same weight, and had

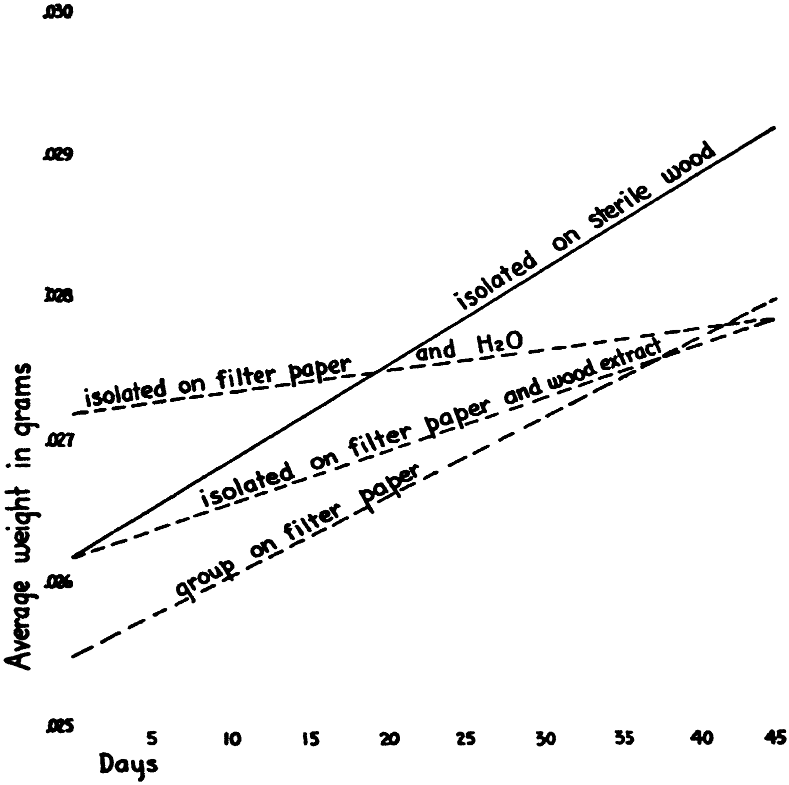

Fig. 4

nearly 50 per cent casualties, caused by excess fungus growths. It is noteworthy that the individuals on sound wood, even though isolated, gained more than the group left together on filter paper. Although this experiment was short, the fact that the termites made differential gains on these diets, even when isolated, tends to substantiate the other results.

CONCLUSIONS

1. A diet of wood as opposed to one of nearly pure cellulose in the form of Whatman's filter paper No. 44, has a consistent, although at times not very pronounced, accelerating effect on the growth rate of *Termopsis*.

2. A filter paper diet, when administered to early instars, inhibits the development of wing pads.

3. One one-thousandth molal solutions of potassium nitrate, calcium nitrate, and ammonium nitrate added to filter paper have a slightly accelerating effect on the rate of growth.

4. Sound wood, previously sterilized, seems to be more nutritious than decayed wood.

5. Filter paper moistened with sterile, aqueous extract of Douglas fir wood gives a growth curve midway between that for filter paper moistened only with H_2O and that for wood.

6. One commonly eaten species of fungus added to filter paper proved of no apparent nutritional value.

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